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OBSERVATIONS ON THE MECHANISM OF THE REACTION BETWEEN FORMALDEHYDE AND SERUM PROTEINS[1]

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That formaldehyde effects profound changes in the protein molecule is, of course, well known, for the use of formaldehyde as a tanning, fixing, and hardening agent depends upon its property of "denaturing" proteins. One phase of its denaturing action—that is, the effect of formaldehyde on the solubilities of the proteins of blood serum in ammonium sulphate solutions—was described in a previous paper (*4*)[2]. The present paper reports the results of a study concerning the mechanism of the changes which take place in the proteins of formolized serums.

In order to establish continuity with the preceding paper it seems desirable to give the following brief summary of the pertinent findings previously reported: (1) The addition of formaldehyde to blood serum caused a progressive decrease in the solubility of the proteins in ammonium sulphate solution. In that respect, the albumin behaved as if changed to pseudoglobulin; the pseudoglobulin as if changed to euglobulin; and the native and formed[3] euglobulins, in their turn, as if changed to proteins of still lower solubility. (2) With a given serum the rate of transformation was proportional to the concentration of formaldehyde, and with sufficient formaldehyde proceeded to the complete disappearance of the albumin and pseudoglobulin fractions. (3) The addition of sufficient formaldehyde to serum resulted in the formation of a gel. With given concentrations of formaldehyde the rapidity of gelatification was determined by (*a*) the concentration of the proteins and (*b*) the concentration of the salts.

The study of the mechanism of the reaction by which the more soluble serum proteins are converted by the action of formaldehyde into less soluble forms was directed along the following lines: (1) The effect of formaldehyde on the precipitation limits of pure globulins and albumins; (2) the order of the reaction which results in the conversion of the more soluble proteins into euglobulins; (3) the effect of temperature on the velocity of the reaction; (4) the effect of the addition of formaldehyde on the titrable acidity; and (5) the effect of formaldehyde on the hydrogen-ion concentration of the serum.

THE EFFECT OF FORMALDEHYDE ON THE PRECIPITATION LIMITS OF PURE GLOBULINS AND ALBUMINS

Schwarz (*15*) treated serum albumins and globulins with formaldehyde and found that the treated albumins and globulins were precipitated by salts which did not precipitate them before treatment. He did not undertake an exact study of the change which occurred, nor did he state the concentration of salts and proteins which he employed. In order to obtain more specific information the following experiment was made.

EXPERIMENT I

Euglobulins, pseudoglobulins, and albumins were separated as precipitates from a clear, normal, hog-blood serum

[1] Received for publication April 19, 1924—issued February, 1925.

[2] Reference is made by number (italic) to "Literature cited," p. 481–482.

[3] Native euglobulins = the protein of native serum which is insoluble in one-third saturated ammonium sulphate solution. Formed euglobulins = the proteins insoluble in one-third saturated ammonium sulphate solution formed by the action of formaldehyde on the more soluble proteins.

by appropriate treatment with ammonium sulphate. The precipitated fractions were dissolved separately and the salts removed by dialysis. After dialysis, NaCl in an amount sufficient to make a concentration of 0.9 per cent was added to each dialyzed fraction. As the volumes of these fractions were unequally increased by dialysis, identical concentrations of the separate proteins unfortunately were not obtained. The solutions thus obtained were examined on the day before the formaldehyde was added, and on the tenth, eighteenth, sixtieth, and ninety-fifth days thereafter. On the sixtieth day the amount of formaldehyde in the albumin fraction was increased to 0.37 per cent and on the ninetieth day to 0.74 per cent. The results of the examination are reported in Table I.

It is to be noted that the concentration of proteins in the albumin fraction was lower than in any of the other fractions. This test suggests that albumins, in the presence of formaldehyde, probably pass through the stage of solubility corresponding to pseudoglobulins before the stage corresponding to euglobulins [4] is reached.

This experiment has been repeated several times under practically the same conditions, and in each instance the results confirmed those presented above. The several experiments of this study may be summarized as follows: In the presence of 0.18 per cent formaldehyde pure native pseudoglobulins behaved as if they were converted into euglobulins, and pure native albumins as if converted into pseudoglobulins, but euglobulins were not produced from albumin solutions

TABLE I.—*The effect of formaldehyde on the precipitation limits of pure globulins and albumins*

Protein fraction	Time of contact	Grams per 100 c. c.		
		Euglobulins	Pseudoglobulins	Albumins
	Days			
Euglobulin	[a] 0	1.86	0.45	0.00
	10	Gelled.		
Pseudoglobulin	[a] 0	.08	2.78	.00
	10	.50	2.36	
	18	1.60	1.26	
	60	2.12	.74	
Albumin	[a] 0	.00	.15	1.73
	10	.00	.36	1.52
	[b] 60	.00	.80	1.08
	[b] 90	No examination was made.		
	95	.52	.98	.38

[a] Before the formaldehyde was added.
[b] The amount of formaldehyde was increased.

The euglobulin fraction before treatment contained a small amount of native pseudoglobulins and in this fraction gelatification occurred by the tenth day. In the pseudoglobulin fraction there was an increase in the formed euglobulins throughout the duration of the experiment. In the albumin fraction with 0.18 per cent of formaldehyde there was a progressive increase in the protein designated as pseudoglobulins, but even after 60 days there was no appearance of formed euglobulins; however, on increasing the formaldehyde in this fraction to 0.74 per cent on the ninetieth day there was a production of formed euglobulins. containing only 0.18 per cent formaldehyde. With greater amounts of formaldehyde, euglobulins were produced from albumins.

THE ORDER OF THE REACTION AS DETERMINED BY THE VELOCITY OF THE REACTION

In the paper previously mentioned, it was indicated that the velocity of the reaction involved in the formation of euglobulins in formolized serum was controlled by the concentration of the reacting substances, namely, the proteins of the serum on the one hand and the formaldehyde on the other. This

[4] Throughout this paper the unmodified term "euglobulins" is used to refer to any protein which is precipitable by one-third saturated ammonium sulphate whether it was originally present in the serum or was formed by the action of formaldehyde on serum proteins.

suggested the possibility of determining the order of the reaction. Accordingly, an experiment was performed for the purpose of measuring the extent of transformation of the more soluble proteins into formed euglobulins after they had been subjected to the action of formaldehyde for definite periods of time. From the resulting figures the velocity constant was calculated by the use of the monomolecular formula,

$$K=\frac{l}{t}\log_n\frac{A}{a-x}.^{5}$$

The values for K thus obtained were so irregular that it was concluded that the reaction was either not of the first order or that the simultaneous occurrence of side or consecutive reactions was obscuring the results. In order to avoid complexities of this character, recourse was had to Ostwald's (*9*) expedient of determining the order of a reaction. As employed in this study, this involved dilution of the original solution and the determination of the time required for the euglobulins in the diluted and undiluted solutions, respectively, to be increased by a definite percentage, or inversely, the time required for the noneuglobulin proteins of each to undergo a given amount of transformation into euglobulins.

As stated by Bigelow (*2, p. 361*), this method depends upon the fact that—

> if a reaction is of the first order, the time required to reach a given stage is not altered by altering the original concentration. * * * If it is of the second order the time required to reach a given stage is inversely proportional to the original concentration. * * * If it is of the third order, the time required to reach a given stage is inversely proportional to the square of the original concentration.

Thus, the time required for a given amount of work to be done in concentrated solutions on the one hand and half concentrated on the other may be expressed by the following ratios: For first-order reactions as 1 : 1; for second-order reactions as 1 : 2; and for third-order reactions as 1 : 4.

In formolized serum at least two proteins are converted into euglobulins, so at least two reactions must progress either simultaneously or consecutively. Therefore, since only one product, euglobulin, is formed and since the ratio between the time required for a given amount of work to be done in the concentrated and half-concentrated solutions is determined by comparing the rate of formation of this one product in the two solutions, the reactions involved in the transformation of the more soluble serum proteins into euglobulins are to be regarded as either side or consecutive reactions. Mellor (*7, p. 75*) states that—

> a reaction may be really compounded of two or more side reactions of the same order and yet have the same formal integrated equation as a normal una-bi- * * * molecular reaction.

Ostwald's method is designed to overcome the disturbing influence of side or consecutive reactions.

Provided that the reactions are side reactions and all belong to the same order, one of the above-named ratios should apply. On the other hand, if they belong to different orders and occur simultaneously, some intermediate ratio should apply, while if they are of different orders and occur consecutively, a change between the earlier and later ratios should take place. With these ideas in mind, the method of Ostwald was applied to the reaction which results in the formation of euglobulins in formolized serum.

EXPERIMENT II

To 190 c. c. of horse-blood serum of known protein content, and previously heated to 38° C., 10 c. c. of a formaldehyde solution, which contained 3.74 gm. of formaldehyde, were added. Simultaneously, to a second 190 c. c. of the same serum, 200 c. c. of a 0.9 per cent NaCl solution and 10 c. c. of the same formaldehyde solution (all previously heated to 38° C.) were added. Both portions were held at 38° C.; 10 c. c. samples of the first formolized serum and 20 c. c. of the second were withdrawn at frequent intervals, diluted to 200 c. c., primarily to stop the reaction, and the euglobulins determined at once. All determinations were made in duplicate. From the results obtained at each interval the amount of the more soluble proteins which had not been converted into euglobulins was calculated by subtracting the euglobulins found at the given time from the proteins originally present. The results are shown in Figure 1.

The time required for the more soluble proteins of the two serums to undergo a given degree of change was determined from Figure 1 by interpolation and is shown in Table II.

[5] For a full discussion of this method of determining the order of the reaction, known as the method of integration, any treatise of theoretical or physical chemistry may be consulted. In the equation for the first order reaction, K=a constant, known as the velocity constant; A=the original amount of the substance present; and X=amount of A transformed in time, t; $\log_n$=natural logarithm.

TABLE II.—*The ratio of the rate of the reaction in undiluted to the rate of reaction in diluted formolized serums held at a temperature of 38° C.*

Proportion of noneuglobulin protein converted	Minutes required to effect conversion		Ratio
	Undiluted serum	Diluted serum	
25	8½	16	1 : 1.9
33⅓	11	21	1 : 1.9
50	17	36	1 : 2.1
60	24	73	1 : 3.0
66⅔	33	107	1 : 3.2

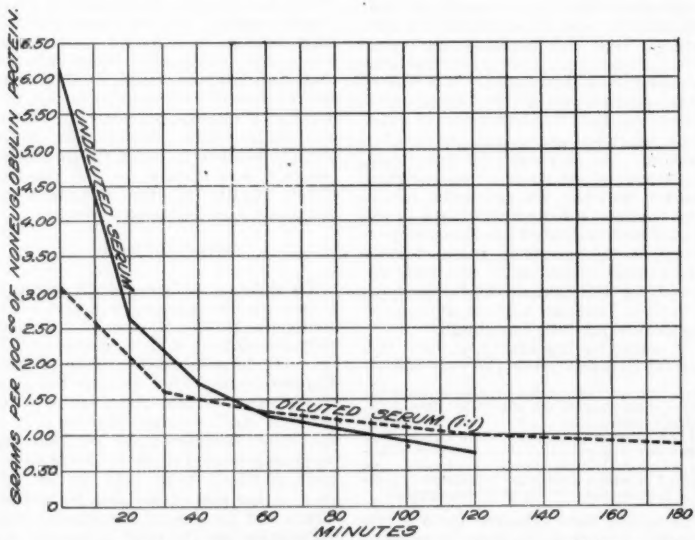

FIG. 1.—The rates of transformation of the more soluble serum proteins into euglobulins in undiluted and diluted formolized serums

By comparing the time required for a given amount of work to be done in the concentrated and in the half-concentrated serums (Table II), it will be seen that in the earlier stages of the reaction the ratio between the times required for a given amount of transformation to take place in the proteins of the concentrated and half-concentrated serums is approximately as 1 : 2. When the noneuglobulin proteins had been reduced by one-half an increase in this ratio takes place. Essentially the same results have been obtained in several other experiments. The probable cause of the change in this ratio will be discussed at the end of this section. It is regretted that pressure of other work did not permit an extension of this study to include solutions of pure pseudoglobulins and albumins; the results obtained, however, point rather strongly to the probability that the reactions involved in the transformation of the more soluble serum proteins of formolized serum consist, at least in the early stages, of reactions of the second order.

Confirmation of the probability of the reactions belonging to the second order was supplied unexpectedly by the following facts: Of the several formulas employed to determine whether a reaction is of the second order, one of them,

$$\frac{1}{t} \cdot \frac{x}{a-x} = ak,$$

applies only when the reacting substances are present in equimolecular proportions. In this formula t=time, a=amount of substance originally present, and x=amount of substance converted. In order to determine the values of t, a, and x a definite amount of formaldehyde was added to a serum of known composition. This was kept at a temperature of 25° C. Samples were taken at definite periods, the euglobulins determined, and from the values so obtained the velocity constant ak was calculated. The results are shown in the following Table:

TABLE III.—*The velocity constant of the reaction taking place in serum containing 0.74 per cent formaldehyde and kept at a temperature of 27° C*

[a]t	[b]x	[c]$a-x$	$ak=\frac{1}{t}\cdot\frac{x}{a-x}$
0	0.00	6.00	
15	.92	5.08	0.012
30	1.46	4.54	.010
45	1.87	4.13	.010
60	2.55	3.45	.012
120	3.30	2.70	.010
240	4.28	1.72	.010
360	5.00	1.00	.013
Average			.011

[a] t=time of contact.
[b] x=noneuglobulin protein coverted to euglobulins.
[c] a=original noneuglobulin protein.

The value for ak in Table III is a constant within the range of experimental error. Data from six other experiments in which undiluted serums were used, and in which t, x, and a had been determined were available. From these data ak was in each case calculated and the values so found were in each case constant within the range of experimental error. However, calculations of ak from the data obtained when diluted serums were used yielded decreasing instead of constant values. Thus, in the undiluted serum of Experiment II ak was found to have a value of 0.066 throughout the reaction, but in the diluted serum the values calculated for ak decreased from 0.033 to 0.015 in two hours. This suggests that the reaction in the diluted serum was approaching equilibrium, and this suggestion would account for the fact that a constant ratio was not obtained in Experiment II.

The fact that constant values were obtained for ak in the experiment reported in Table III and that a ratio of 1 : 2 was obtained in the early part of Experiment II points strongly to the probability that the reactions which take place in formolized serum belong to the second order even though the experimental data available may not absolutely establish such a conclusion.

THE EFFECT OF TEMPERATURE

The effect of temperature on the velocity of the reactions which result in the production of euglobulins in formolized serum was determined by the following experiment:

EXPERIMENT III

A horse-blood serum of known composition was divided into two portions, one of which was warmed to a temperature of 39° C. and the other to a temperature of 29°. When the serums had reached these respective temperatures a formaldehyde solution previously heated to the respective temperature was added to each in such amounts that each serum contained 1.85 per cent formaldyhyde. The serums were maintained at the respective temperatures for three hours, samples of each being taken at intervals for the determination of euglobulins. From the amounts of euglobulins found at each determination the amount of noneuglobulin protein remaining unchanged at each instant was calculated by subtracting the amount of euglobulins found from the total proteins present. The results are shown in Figure 2. From this figure the time required for given amounts of work to be done in the two serums was determined by interpolation and is shown in Table IV.

TABLE IV.—*The temperature coefficient of the reactions taking place in formolized serums as determined by comparing the times required for a given amount of work to be done in serums containing 1.85 per cent of formaldehyde and kept at 29° and 39° C., respectively*

Proportion of noneuglobulin protein converted	Minutes required to effect conversion		Temperature coefficient
	Serum at 39° C.	Serum at 29° C.	
25	7	18	2.60
33⅓	10	20	2.90
42	15	43	2.87
50	21	63	3.00
66⅔	41	140	3.41
70	65	180	2.80
Average			2.93

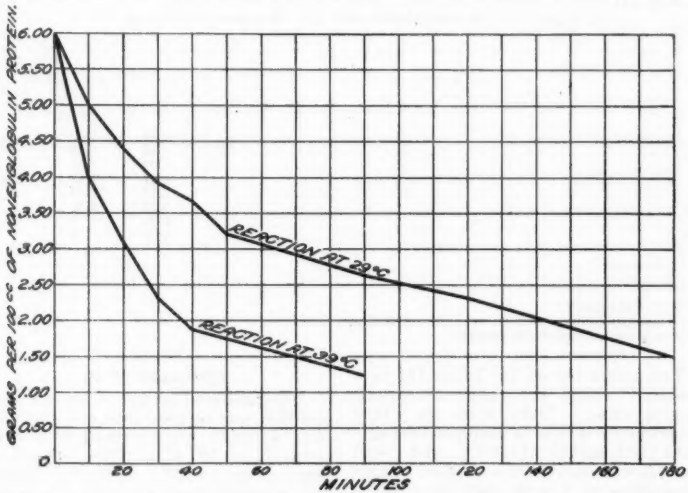

FIG. 2.—The rate of reaction in serums containing 1.85 per cent formaldehyde and kept at 29° and 39° C., respectively

An inspection of these results shows that the temperature coefficient of this reaction was approximately 3. This relatively high temperature coefficient eliminates the possibility of mere physical changes being responsible for the alteration in solubility brought about in serum proteins by the addition of formaldehyde, since the temperature coefficients of physical reactions are less than 2 (*13, p. 216*).

It is believed that the foregoing results show that the reaction in this experiment had a temperature coefficient of 3. However, in order to determine whether the same temperature coefficient could be obtained by comparing the velocity constants of the two reactions, the values for these were calculated by the use of the bimolecular formula in the manner previously described. The results are shown in Table V.

It will be noted in Table V that fairly constant values for ak were obtained for both serums, and that the value for ak obtained in the case of the serum kept at 39° C. is three times as great as for the serum kept at 29°. Thus by this method a temperature coefficient of 3 is also shown.[6]

[6] The serum upon which Table III is based and the serum shown in Table V as having been held at 29° C. both contained the same amount of protein and were held at about the same temperature. The two serums contained different amounts of formaldehyde; nevertheless, velocity constants, although not identical, were very similar. This suggests that the rate of reaction in formolized serums, provided an excess of the aldehyde is present, depends more upon the concentration of the proteins than upon that of the formaldehyde. There is also a suggestion that the formaldehyde itself does not enter the reaction of which the velocity constant was determined. These suggestions are in harmony with the observation that the addition of formaldehyde in excess may retard the rate of gelatification of serums.

TABLE V.—*The temperature coefficient of the reactions in formolized serums, determined by comparing the velocity constants of the reactions taking place in serums containing 1.85 per cent formaldehyde and kept at 39° and 29° C., respectively*

t	Serum kept at 39° C.			Serum kept at 29° C.		
	x	$a-x$	ak	x	$a-x$	ak
0	0.00	6.00		0.00	6.00	
10	1.98	4.02	0.049	.98	5.02	0.019
20	2.90	3.10	.047	1.60	4.40	.018
30	3.69	2.31	.053	2.07	3.93	.017
40	4.14	1.86	.055	2.33	3.67	.016
50	4.27	1.73	.049	2.77	3.23	.017
60	4.37	1.63	.044	2.93	3.07	.016
90	4.76	1.24	.043	3.37	2.63	.014
120				3.68	2.32	.012
180				4.53	1.47	.017
Average			.048			.016
Coefficient			3.0			1.0

THE TITRABLE ACIDITY OF FORMOLIZED SERUMS

Schiff (*14*) found that when formaldehyde was added to a solution of gelatin the titrable acidity increased as more formaldehyde was added. He also added unstated amounts of formaldehyde to albumin solutions and found that an immediate and relatively great increase in acidity was followed after 24 hours by slight decreases. Many observations upon the effect of formaldehyde on the titrable acidity of serums have been made by the writer, but as the results of all have been in accord, only one will be described.

EXPERIMENT IV

A clear, horse-blood serum was divided into four portions of 100 c. c. each. To these four portions there was added, respectively, ½ c. c., 1 c. c., 3 c. c., and 6 c. c. of a formaldehyde solution (37.34 per cent). Samples (10 c. c. each) were removed from each portion at the intervals shown in Table VI and titrated to a slight pink with N/10 NaOH, using phenolphthalein as an indicator.

These results show that following the addition of formaldehyde to a serum there was a rapid increase in titrable acidity. The speed and extent of this change were related to, but not proportional to, the amount of formaldehyde added. The primary increase in titrable acidity was followed by a gradual decline. The observations were discontinued at the ninety-sixth hour, so the full extent of the decline was not determined. Identical results have been obtained in a number of other experiments. However, in one serum which before treatment required about 20 c. c. of N/10 NaOH per 100 c. c. for neutralization, no increase in acidity was observed following the addition of formaldehyde, but only a constant decline.

TABLE VI.—*The titrable acidity of formolized serums*

Time of contact	N/10 NaOH required to neutralize 100 c. c. of serum			
	With ½ c. c. formol	With 1 c. c. formol	With 3 c. c. formol	With 6 c. c. formol
	C. c.	*C. c.*	*C. c.*	*C. c.*
Before treatment	8	8	8	8
3 minutes	19	30	39	42
15 minutes	23	28	38	39
1 hour	21	26	33	37
3 hours	13	20	25	25
24 hours	9	11	20	20
48 hours	6	11	17	19
96 hours	5	10	15	18
124 hours				Gelled.

The most plausible explanation of the decline in acidity seems to be that this is due to neutralization of the originally free, and later liberated, COOH groups by basic groups which have not yet been attacked by formaldehyde. This explanation depends upon the possibility of basic groups existing in the presence of formaldehyde. That such a possibility exists has been shown by Schiff (*14*), who reported that relatively few NH_2 groups of proteins are combined with formaldehyde, and by Proctor (*10, p. 145*), who has shown that the reaction between amino-acid anhydrides and formaldehyde, and also between histidine and formaldehyde, is irregular; that is, not all of the NH_2 groups are combined.

If the assumption that the decline in acidity of formolized serums is due to a neutralization of the COOH groups of the protein by NH_2 or other basic N groups be accepted, then the reason for the applicability of the formula

$$ak=\frac{1}{t}\cdot\frac{x}{a-x},$$

which applies only when the reacting substances are present in equimolecular proportions, becomes apparent: proteins are composed of amino acids; and practically all amino acids contain basic and acid groups in equimolecular quantities.

THE P_H VALUE OF FORMOLIZED SERUMS

The results obtained in the titration studies suggest, of course, that the reactions which result in the transformation of the more soluble proteins of formolized serums into less soluble forms may be caused by changes in the hydrogen-ion concentration induced by the presence of added formaldehyde. The following two experiments bear on that question.

EXPERIMENT V

A hog-blood serum was divided into two portions. To one, 0.09 per cent of formaldehyde was added in the form of a solution of formaldehyde, and to the other 0.5 per cent of phenol was added. The euglobulins and the P_H value [7] of the serums were determined at the intervals shown in Table VII.

It will be noted that following the addition of formaldehyde and phenol, respectively, an initial decrease in the P_H value took place, and that this was in each sample followed by an increase. In the formaldehyde-treated fraction the euglobulins increased 124 per cent, while the phenol fraction increased but 24 per cent. This suggests that the changes in the solubilities of the proteins of formaldehyde-treated serums are not due solely to changes in the P_H value.

EXPERIMENT VI

A horse-blood serum was divided into two portions, to one of which 0.74 per cent and to the other 1.85 per cent of formaldehyde were added. The P_H value and the titrable acidity of these serums were determined at the intervals and with the results shown in Table VIII.

TABLE VII.—*The effect of formaldehyde and phenol on the P_H value and euglobulin content of hog-blood serum*

Added	Time of contact	P_H	Euglobulins (grams per 100 c. c.)
Nothing	Fresh	8.4	1.41
0.09 per cent formaldehyde	1 day	8.0	1.81
	1 month	8.3	3.16
0.05 per cent phenol	1 day	8.0	1.60
	1 month	8.5	1.75

[7] Determined colorimetrically by Dr. F. W. Tilley, of this division.

TABLE VIII.—*The changes in the titrable acidity and P_H values of formolized horse-blood serum*

Time of contact	Grams of formeldehyde added per 100 c. c. of serum			
	0.74		1.85	
	P_H	Acidity*	P_H	Acidity*
Before treatment	6.6	8.0	6.6	8.0
3 minutes	6.1		5.9	
1 hour	6.1	36.0	5.9	38.0
6 hours	6.1	28.0	5.8	30.0
24 hours	6.1	15.0	5.6	18.0
72 hours	6.0	15.0	5.6	15.0

* C. c. N/10 NaOH required to neutralize 100 c. c. of serum.

In this experiment the P_H value of the serum decreased and the titrable acidity increased on the addition of formaldehyde to the serum, and the changes in each were greater in extent when greater amounts of formaldehyde were added. However, while the immediate increase in titrable acidity was followed by a decline, no converse change occurred in the P_H value, which tended to decrease throughout the duration of the experiment. Attention is called to the fact that the serums reported in these two tables had different P_H values. The only explanation that can be offered is that the two serums were different, that they were derived from different species of animals, and that the serum with the lower P_H value had been kept longer before the experiment was begun. From the results of Experiments V and VI it is apparent that the addition of formaldehyde to a serum results in a decrease of the P_H value of the serum. It is also indicated that the change in P_H value of serums after formolization is not the sole factor controlling or inducing the transformation of the more soluble proteins into euglobulins.

MISCELLANEOUS OBSERVATIONS

In addition to the observations recorded in the preceding sections of this paper, a number of other observations regarding the behavior of the protein, including the factors influencing that behavior, have been made. It is not believed that the later observations have been substantiated sufficiently to warrant their presentation in detail. However, a few of these observations will be discussed briefly because they are considered of interest and importance.

Schwarz (*15*) found that when formaldehyde was added to serum a part of the aldehyde immediately, while another part gradually, combined. Similar results have attended experiments with formolized serums made in the hope of gaining information regarding, first, the relation between the rate of disappearance of uncombined formaldehyde and the rate of production of euglobulins, and, secondly, the relations between the amount of formaldehyde added and the amount combined. Thus, in several experiments 1.85 per cent of formaldehyde was added to serums. Following the addition of the aldehyde, the serums were examined at intervals for the purpose of determining the proportion of free formaldehyde which had disappeared from solution. In every test conducted in this way it was found that (*a*) a small amount, usually less than 0.33 per cent, disappeared, probably combined, within the first few minutes of the test, and (*b*) no increase in this amount occurred within the first five or six hours of the experiment. When the amount of aldehyde found to have disappeared in the first few minutes of the test was small, i. e., less than 0.20 per cent, and when the examination was repeated at the end of 24 hours, there was in some cases an indication that a slight additional amount of free formaldehyde had disappeared.

In regard to a relation between the amount of formaldehyde added to the amount combined: In a series of 12

experiments there was, with one exception, a greater amount of formaldehyde combined when a greater amount was added.

That the presence of salts is necessary for the formation of gels has been shown in the preceding paper. While the part played by salts has not been investigated, it has been noted that a greater part of the noneuglobulin proteins of the serum may be converted into euglobulins within a few hours and the serum yet fail to gel within many days. If salt is added to such formolized serums, they gel within a short time. This would suggest that the presence of salt, although necessary for gelatification, is not necessary for the transformation of the more soluble proteins into euglobulins.

DISCUSSION

It has been shown by Moll (*8*), Banzhaf (*1*), and others that an exposure of serum to a temperature of 50° to 60° C. for more or less prolonged intervals results in the transformation of albumins into pseudoglobulins and the latter into euglobulins. If the temperature to which the serum is exposed is sufficiently high, coagulation, of course, results. On the addition of formaldehyde to serum a similar transformation of the proteins occurs. If the concentration of the formaldehyde, other factors not preventing, is sufficiently high, gelatification results.

According to Chick and Martin (*3*), the reaction involved in the heat coagulation of protein increases in velocity with increase of temperature and they give 8 as the temperature coefficient of the heat coagulation of hemoglobin. The experiments reported in this paper indicate that the transformation by formaldehyde of the more soluble serum proteins into euglobulins increases in velocity with increase of temperature. The temperature coefficient is 3.

Mann (*6, p. 318*) states, and Sørensen and Jürgensen (*16*) confirm him, that any protein solution containing salts, acids, or bases becomes more alkaline on being coagulated. Although the addition of formaldehyde to serum results in a primary increase in acidity, this is, as has been shown herein, always followed by a decline in titrable acidity, and this decline may continue until the serum is more alkaline than it was in the beginning.

It is well known that native serums diluted with five or more parts of water do not coagulate on being heated, but if salt is added to such diluted and heated serums a coagulum is produced. This can be explained by the fact that heat coagulation of proteins consists, as shown by Chick and Martin and quoted by Robertson (*12, p. 307*) of two processes, (*a*) denaturation and (*b*) agglutination, and this occurs only in presence of salt. It has been shown in this study that the addition of formaldehyde to serum causes (*a*) denaturation, i. e., the conversion of the more soluble proteins into less soluble forms, and (*b*) gelatification, which latter condition, so far as the writer's observations go, occurs only in the presence of salts.

Chick and Martin (*3*) have shown that heat coagulation of proteins is not an instantaneous process, but that it proceeds with a definite velocity. In the work reported herein, it has been shown that the transformation by formaldehyde of the more soluble serum proteins into euglobulins, the end result of which is gelatification, is not an instantaneous process but that it also proceeds with a definite velocity.

From the foregoing it seems apparent that an analogy exists between the mechanism of heat coagulation on the one hand and of formal-gelatification on the other. However, at a certain point the analogy ceases, for while the reaction concerned in the heat coagulation of serum belongs to the first order, the reactions concerned in formol-gelatification seem to belong to the second order.

Robertson (*12, p. 309*) states that heat coagulation "is essentially a phenomenon of dehydration of which the first stage, that of internal neutralization through the loss of the elements of water from the end $-NH_2$ and COOH groups, probably corresponds to the phenomenon of denaturation while the subsequent or simultaneous polymerization of these anhydrides leads to the formation of particles so large as to assume the properties of matter in mass, i. e., flocculi." He states that Mann, Sutherland, Hofmeister, and Pauli have expressed similar views.

The points of similarity and contrast between the mechanism of the reactions which occur during heat coagulation and formol-gelatification have suggested a possible explanation of the latter phenomenon. It will be recalled that an immediate increase in titrable acidity followed the addition of formaldehyde to serum. This may be considered as the first stage in the reaction and may be explained, perhaps, by the occurrence of reactions resembling the reaction which takes place between amino acids and formalde-

hyde. It is, of course, well known that the addition of formaldehyde to amino acids results in a reaction which may be represented by the equation:

$$NH_2-R-COOH+CH_2O \rightleftarrows CH_2N-R-COOH+H_2O$$

Thus, the basic group of the amino acids is attacked by formaldehyde and so loses its pronounced basic character, while the acid character of the amino acid is correspondingly increased. It seems plausible to attribute the primary increase in acidity, which results from the addition of formaldehyde to serum, to reactions of that type; that is, in the first stage of the reaction in formolized serum, the formaldehyde may attack certain basic groups of the protein molecule as a result of which the protein molecule itself becomes acid in character. Such a reaction may be represented by the equation:

$$NH_2-R-COH=N-R-COOH+CH_2O \rightleftarrows CH_2N-R-COH=N-R-COOH+H_2O$$

Following the initial increase in titrable acidity, a gradual decline was found to occur. This may be considered as the second stage of the reaction. It is well known that NH_2- groups are not responsible for all the acid-combining capacity of the protein molecule, for all the NH_2- groups of the molecule may be removed by the action of nitrous acid and the molecule will still combine with acid. Because of this and similar facts, the reserve acid-combining capacity of the protein molecule has been ascribed by Robertson (*13, p. 156*) to the presence of enol, $-C(OH)=N-$, or similar linkages. To the nitrogen of this linkage is attributed the possession of two latent valencies, one positive and one negative. When the N is trivalent these two valencies neutralize each other; when pentavalent, they are capable of neutralizing a negative and positive radical, respectively.

$$-C(OH)=\overset{\overset{H}{|}}{N}-\overset{|}{Cl}$$

It is possible that the decrease in titrable acidity observed in formolized serums is due to reactions between such internal groups, on the one hand, and acid protein molecules, formed in the first stage of the reaction, on the other. The following formula represents such a possible combination:

$$CH_3-N-R-C(OH)=\overset{\overset{H}{|}}{N}-R-COOH \quad \overset{|}{OOC}-R-N=(HO)C-R-N-CH_3$$

That a neutralization of the original and liberated $-COOH$ groups of formolized serums occurs seems assured. That the reactions described above offer a possible mechanism seems plausible. If this neutralization could be brought about by a combination of the acid groups with unattacked basic groups of the protein, then this neutralization could occur not only between adjacent basic and acid groups of the same molecule but between such groups of adjacent molecules. This would account for the finding that the reaction apparently belongs to the second order. We should thus have a polymerization which would be expected to continue until all of the protein molecules which had been attacked by the formaldehyde had united with other affected or unaffected molecules. Thus the proteins would acquire the properties of matter in mass and a network of the protein would extend throughout the serum, the visible result of which would be gelatification. That gels possess a network is accepted, according to Lloyd (*5*), by many as a fact. Reiner and Marton (*11*) have recently attributed the formol-gelatification of serums to polymerization, though without advancing confirmatory evidence.

This explanation of the mechanism of the changes which take place in the proteins of formolized serum is offered simply as a suggestion and with the full realization that the results presented herein are not sufficient to warrant at the present time a final conclusion.

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A BACTERIAL LEAFSPOT OF MARTYNIA[1]

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INTRODUCTION

Martynia louisiana is a coarse spreading annual with large pubescent leaves. The fruit is a capsule with a fleshy, hairy covering and long curved beak, which gives it the common name devil's claw or unicorn plant. It grows as a weed in the warmer parts of central United States and is also cultivated, the young fruits being used for pickles.

In July, 1922, diseased leaves of *Martynia louisiana* (*M. proboscidea*) were sent in by J. G. Lill, of the Office of Sugar Plant Investigations, from Garden City, Kans., (Pl. 1, A) where the plants were growing as weeds in and along the borders of sugar-beet fields. Bacteria were isolated from the leafspots but pure culture inoculations have shown that the organism isolated was not infectious to beets and was in no way connected with the spotting of the beet leaves. Finding no description in literature of a bacterial leafspot of Martynia, the following study of the disease as it occurred on the host plant and of the morphology, physiology, and cultural characters of the causal organism was made.

DESCRIPTION OF LESIONS

The leaves were heavily spotted with lesions mostly round but angular when bounded by veins. The infected tissue is water-soaked, sunken (Pl. 1, A) and translucent. The youngest spots are 1 to 2 mm. in diameter. The translucent centers are not more than 2 mm. in diameter; but narrow, swollen, brown margins, slightly raised above the surrounding tissue, develop as the spots become older. When several spots coalesce they form irregular patches of dry, light-brown tissue a few millimeters to a centimeter or more wide with translucent dots scattered over them. From sections (Pl. 2, A, B, and C) of these lesions bacteria stream in abundance.

ISOLATIONS AND INOCULATIONS

Isolations were made in two ways. Lesions were washed through 12 sterile water blanks, crushed in sterile broth and plates were poured. Other lesions were dipped in 95 per cent alcohol, then into $HgCl_2$ 1–1,000 for two minutes, washed through three sterile water blanks, crushed in sterile broth and plates poured.

Both methods gave practically pure cultures of a white organism which when sprayed on Martynia plants produced typical lesions of the disease (Pl. 1, B). Reisolations from these lesions again gave the same white colonies which again produced the leafspot when sprayed on Martynia.

Inoculations were made by spraying plants both in the field and greenhouse with water suspensions of the causal organism. Check plants were sprayed with sterile water. Inoculated greenhouse plants and checks were held in damp chambers three to four days. Four to six days after inoculation, lesions began to appear as water-soaked dots on the under surfaces of the leaves. Two or three days later the lesions may be a millimeter in diameter and visible on the upper as well as the under surface. The checks remained healthy.

In two greenhouse experiments infection appeared to progress through the veins, many of which, leading from marginal or other lesions, were water-soaked and darkened or more transparent than normal. Petioles collapsed at the point of junction with the blade and later entire leaves and petioles died and dried. From the petioles the infection passed into the stem until entire plants collapsed and dried up. In field experiments infection seemed to pass from growing tips through the stem into the more or less fully developed fruits, causing at first a water-soaked appearance. Later, this tissue turned brown, shriveled, and died

[1] Received for publication June 14, 1924—issued February, 1925.

Bacterial Leafspot of Martynia

PLATE I

(For explanatory legend see p 483)

INOCULATIONS ON OTHER PLANTS

Injured and uninjured leaves of young beet plants were sprayed at two different times with water suspensions of the organism causing leafspot of Martynia. No lesions were produced.

Three attempts to produce infection on young cucumber plants in the greenhouse gave negative results. In one experiment Martynia leaves were sprayed at the same time as the cucumber plants and kept under the same conditions. Typical lesions developed in abundance on the Martynia leaves.

Leaves of the trumpet creeper, *Tecoma radicans* (L.) Juss., were also inoculated by rubbing with a water suspension of the organism. No infection resulted.

THE CAUSAL ORGANISM

MORPHOLOGY

The organism is a short rod with rounded ends, occurring singly or in pairs. Chains occur on 8-day beef peptone agar (Pl. 3, F). Measurements of organisms grown 24 hours on beef peptone agar and stained with gentian violet vary in length from 2.2μ to 1.3μ in length and from 0.7μ to 0.5μ in width, with averages of 1.68μ by. 59μ.

Capsules are present on beef peptone agar. (Plate 3, G). Sporangia and endospores are not formed. The organism is motile, having one to several bipolar flagella (Pl. 3, E).

The organism is Gram-negative.[2]

Smears from a 2-day beef peptone agar culture stained for 5 minutes in steaming carbol fuchsin and dipped for a few seconds in 20 per cent aqueous solution of H_2SO_4 were not acid-fast.

CULTURAL CHARACTERISTICS

Cultures were grown at room temperature unless otherwise noted.

BEEF PEPTONE AGAR SLANTS.—Growth is moderate, filiform, raised, glistening, somewhat contoured. There is a slight odor of decay, consistency is butyrous with traces of viscidity. The growth is white but may have a slightly opalescent tinge, and the agar turns slightly greenish brown. Numbers of small crystals form over and through the agar.

POTATO DEXTROSE AGAR SLANTS.—Growth is abundant, spreading, glistening, somewhat translucent, white by reflected light, but deep cream by transmitted light. Consistency is butyrous and there is no odor.

GELATIN STAB.—Held in the Altmann at about 20° C., growth is best at the top and extends only a few millimeters down along the line of puncture. Liquefaction is stratiform, beginning in 48 hours and completed in about 2 months. The liquefied gelatin becomes slightly fluorescent.

NUTRIENT BROTH.—A thin pellicle is formed with small crystals through it. When slightly jarred, the pellicle hangs down in long loops and strands from these crystals, which tend to stay on the surface. Clouding is moderate, being slightly heavier under the pellicle, and when cultures are undisturbed they often show definite bands of clouding. As cultures grow older the medium assumes a greenish tinge. Sediment is moderate in amount and granular.

NUTRIENT BROTH PLUS NaCl.—In beef extract broth with a P_H 6.9 clouding appeared in 2 per cent and 3 per cent NaCl in 48 hours; in 4 per cent after 4 days; and 5 per cent after 12 days. There was no clouding in 6 per cent or 7 per cent NaCl, but in both there was some bottom growth. Tests made in beef infusion broth with the same P_H gave the same results. Tests made in both infusion and extract broth with a P_H 8.2 gave clouding in 2 per cent and in one case in 3 per cent NaCl. A few chains occurred in 1 per cent NaCl and there were many long chains in 4 per cent and 6 per cent.

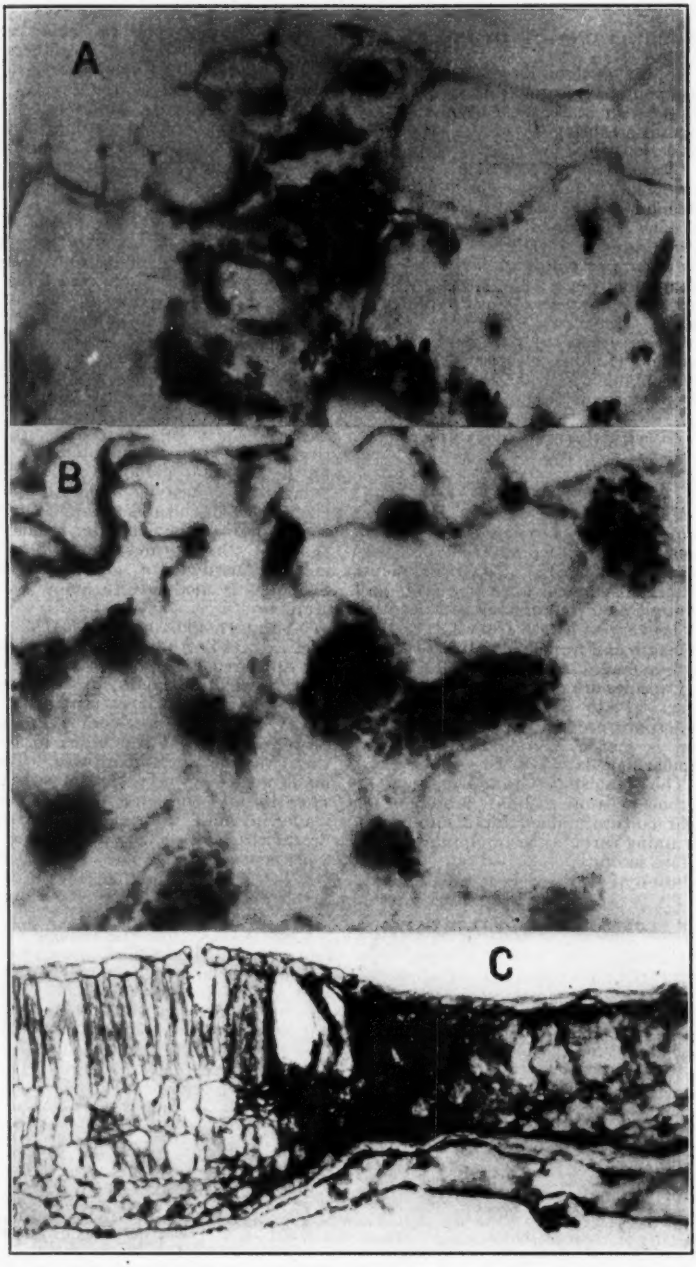
COHN'S SOLUTION.—Clouding is moderate and a thin pellicle forms from which hang large triangular crystals 2 to 4 mm. in length in 3 days. These crystals tend to pull down the pellicles, but crystals still hanging from the surface after 3 weeks are one-half to three-quarters of an inch in length. There was no green fluorescence.

[2] Methods I and II as recommended in "Manual of Methods for Pure Culture Study of Bacteria S. A. B.," were followed.

EXPLANATORY LEGEND FOR PLATE 1

A.—Natural infections on leaf of *Martynia louisiana* (= *M. proboscidea*) collected at Garden City, Kans., Aug. 31, 1922. Photographed by transmitted light Sept. 5, 1922. × 1. Photographic work by James F Brewer.

B.—Leaf of *Martynia louisiana* (= *M. proboscidea*) showing artificial infections. Sprayed Aug. 22, 1923, with water suspension of Culture I in field. Photographed by reflected light Sept. 7, 1923. × 1.



Bacterial Leafspot of Martynia

PLATE 2

(For explanatory legend see p. 487)

USCHINSKY'S SOLUTION.—Clouding is moderate, sometimes heavy with a ring or pellicle and after about a week the medium is light green. After about 8 weeks the cultures are a creamy yellow in color and have pellicles 2 to 4 mm. thick. These pellicles extend in viscid threads or swirls down into the liquid when shaken, but do not sink. There is a small amount of viscid precipitate.

FERMI'S SOLUTION. — Clouding is light. A heavy pellicle forms in a few days and the medium takes on a greenish tinge. After 2 or 3 weeks the pellicle may be 5 mm. deep. After about 8 weeks the cultures are a deep cream color and the pellicle hangs in long viscid strings or loops from the surface. There is no precipitate.

AGAR COLONIES.—Growth in 2 days is moderate, circular, smooth, glistening, raised, 1 to 2 mm. in diameter, with margins entire. By transmitted light fish scale markings become evident in young colonies but later disappear (Pl. 2, A). After 4 to 5 days colonies become umbonate (Pl. 3, C, D). In about a week they are 6 to 10 mm. in diameter in thin sown plates and the medium becomes greenish in tint. Older colonies become translucent.

GELATIN COLONIES.—Growth is moderate, circular, crateriform, the margin is somewhat filamentous, liquefaction is saucer-shaped, internal structure is granular filamentous. Liquefaction is evident in 1 to 2 days and is completed in about a week, depending upon the numbers of colonies on a plate.

BLOOD SERUM.—There is good growth in 48 hours, smooth, glistening, filiform, cream colored, butyrous. In 5 days the blood serum begins to clear under the cultures and becomes slightly depressed. After 2 to 3 weeks the serum is brown and transparent. The slant is thin and depressed under the growth.

PHYSIOLOGY

TEMPERATURE RELATIONS.—The optimum temperature for growth is about 25° C., the maximum about 37°, the minimum about 1.5°. The thermal death point is 49°.

OPTIMUM REACTION AND TOLERATION LIMITS.—The optimum reaction is between +15 and +20 or P_H 6.0 to 6.8. There was growth at +26, P_H 5.4, but no growth at +30, P_H 5.2. There was a trace of growth at −8, P_H 8.9, and no growth at −11, P_H 9.2.

TOLERATION OF ACIDS.—Isolation No. I grew in 0.1 per cent and 0.2 per cent of tartaric, malic, and citric acids, all cultures turning yellowish to bright green. The acids were added to neutral broth giving the following reactions:

Tartaric, 0.1, +10, P_H 7.2; 0.2, +23, P_H 6.0.
Malic, 0.1, +12, P_H 7.0; 0.2, +25, P_H 5.8.
Citric, 0.1, +13, P_H 7.0; 0.2, +26, P_H 5.9.

These acids were also added to uncorrected broth +20, P_H 6.1, to give definite P_H reactions, and cultures grew in the following reactions but not above them:

Tartaric, +29, P_H 5.2.
Malic, +28, P_H 5.4; +33, P_H 5.2.
Citric, +28, P_H 5.7; +33, P_H 5.4.

CHROMOGENESIS.—Nutrient broth, agar, and gelatin are slightly greened. Steamed potato cylinders become cream colored to tan.

INDOL PRODUCTION.—Cultures were grown in a solution of 1 per cent peptone, 0.5 per cent disodium phosphate, and 0.1 per cent magnesium sulphate for 17 days. Tested with H_2SO_4 and sodium nitrite, results were negative.

The same test was made in broth cultures grown for 4 days. Broth cultures 2 weeks old were tested by the Ehrlich and Salkowski methods recommended in the "Manual of Methods." There was no indication of indol production.

H_2S PRODUCTION.—Strips of lead acetate paper were suspended over broth, agar, potato and rutabaga cylinders. There was no discoloration of the paper.

Stabs were made [3] in media containing 5 c. c. of beer extract peptone agar and 5 c. c. of 0.1 per cent basic acetate. Growth at the surface gradually turned light brown, later medium

[3] Following the method recommended by the 1923 "Manual of Methods."

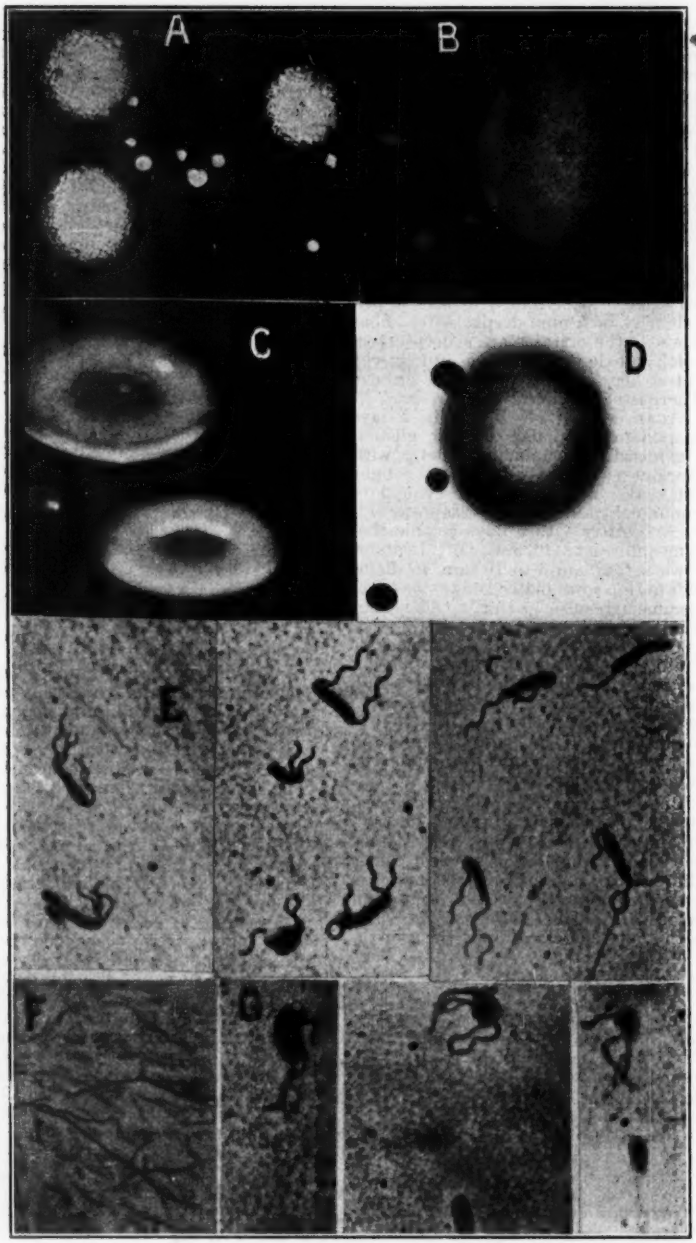
EXPLANATORY LEGEND FOR PLATE 2

Cross sections of infected leaves of *Martynia louisiana* (= *M. proboscidea*). Cut 5μ thick. Carbol fuchsin stain. Photographed Mar., 1924.

A.—Beginning infection. Substomatal cavity filled with bacteria. × 1900.

B.—Beginning infection with bacteria in intercellular spaces. × 1900.

C.—Advancing margin of a 13-day old lesion. Infected tissue has collapsed. Bacteria are most abundant near the margin of the lesion and also appear outside the leaf tissue. × 180.



Bacterial Leafspot of Martynia

PLATE 3

(For explanatory legend see p. 489)

brown, then the agar became light brown in the upper half and finally after 2 months light brown throughout. A small amount of H_2S was produced.

RELATION TO OXYGEN.—Growth was observed in fermentation tubes containing 1 per cent peptone and 2 per cent sugars from which the oxygen had recently been driven off by heating. At the end of a week there was light clouding in the closed arms of tubes containing saccharose, dextrose, and mannit, but not in tubes containing maltose, lactose, and glycerin. In agar shake cultures containing 2 per cent sugars a few small colonies grew in the agar for the depth of half a centimeter in saccharose and dextrose only. The organism is aerobic.

DIASTASIC ACTION.—Streaks were made on beef peptone agar plates containing 0.2 per cent starch. Tested the second day with a saturated solution of iodine in 50 per cent alcohol there was no clear zone around any of the colonies.

Tested after 8 days there was still no cleared zone about the streaks. In some cases a zone 4 to 5 mm. wide was slightly more reddish than the surrounding agar. Twelve plates were tested and on one a fungus contamination had a definite cleared zone about it. There is very little diastasic action.

MILK.—There is evidently a delicate balance between the separation or nonseparation of whey and curd. When separation occurs the curd is evident after 2 days but is always soft. Litmus milk shows very slight acid reaction remaining about the same color as the checks with a slight reddish tinge. A test for rennet gave negative results. Cultures were heated for a half hour at 55° C. and then a few cubic centimeters poured into sterile milk. There was no separation of curd and whey when all the organisms had been killed. The curd is evidently an acid curd. Peptonization becomes evident in 2 to 3 days, progressing in definite bands, the clearest at the top. Peptonization is completed in about 2 weeks. Reduction of litmus begins in about 5 days and is complete in 8 to 14 days. When cultures containing reduced litmus are killed by boiling, the color returns a deeper shade than the checks but neither red nor blue. Reduction of methylene blue in milk is completed in 4 to 5 days. The blue color returns after the organism has been killed by heating the reduced culture. After several months milk turns amber to deep brown and becomes gelatinous or has gelatinous lumps suspended in the liquid.

NITRATE REDUCTION.—No gas is produced. Two tests for nitrites were made in nitrate broth cultures with sulphanilic acid and a-naphthylamine in 5 N acetic acid. Cultures showed good growth. Results were negative during the first few days but showed definite reduction after 2 to 3 weeks. Two tests for nitrites in the synthetic nitrate medium KNO_3 1 gm., K_2HPO_4 0.5 gm., $CaCl_2$ 0.5 gm., glucose 10 gm., distilled water 1,000 c. c. gave positive results. Reduction of nitrates is evident in this synthetic medium in 24 hours. There was no reduction in the checks.

FERMENTATION.—Tests were made in media containing 1 per cent peptone and 2 per cent carbohydrate or one-half per cent peptone and 1 per cent carbohydrate. Gas was not formed. Hydrogen-ion concentrations were determined colorimetrically and the results are given in Table I. Lactose showed no acid reaction, although tests were made after 1, 2, 3, 5, 6, 10, and 16 days, nor were acids detected from raffinose, maltose, mannit, rhamnose, or glycerin. Acid is produced from dextrose, sucrose, galactose, and arabinose.

LITMUS SUGAR AGARS.—Litmus showed reddening in dextrose, galactose, and arabinose after 24 hours; in sucrose in 3 days; in levulose a brownish red in 4 days. Mannit also showed a trace of this browning after 3 days, and when a month old all tubes were slightly redder than the checks. Lactose and maltose were bluer than the checks at the end of a week, and in 11 days the upper parts of cultures of arabinose were turning blue. In from 11 to 20 days partial reduction occurs in sucrose, maltose, levulose, galactose, arabinose, and glycerin.

EXPLANATORY LEGEND FOR PLATE 3

A.—Culture I, 2-day colonies on beef peptone agar. Photographed Mar. 9, 1923. × 10.
B.—Culture II, 3-day colony on beef peptone agar. Photographed Mar. 10, 1923. × 10. Showing the thin margins of young colonies. Transmitted light. Also buried and bottom colonies.
C.—Culture II, 8-day colonies on beef peptone agar. Photographed Mar. 15, 1923. × 10. Showing depressed centers. Reflected light.
D.—Culture II, 5-day colonies on beef peptone agar. Photographed Mar. 12, 1923. × 10. Transmitted light. Showing thin depressed centers and raised heavier borders.
E.—Culture I, 1-day beef peptone agar. Casares-Gil stain. Dec. 15, 1923. × 1900.
F.—Culture I, smear from 8-day beef peptone agar slant. Ribbert's capsule stain. Chains. Photographed Jan. 22, 1924. × 1400.
G.—Culture I, 2-day beef peptone agar cultures. Casares-Gil stain. Capsules. Photographed Jan. 24, 1924. × 1900.

TABLE I.—*Hydrogen-ion concentration of carbohydrate media inoculated with Culture I and compared with uninoculated controls*

Inoculation age (days)	Raffinose		Sucrose		Lactose		Maltose		Mannit		Dextrose		Galactose		Arabinose		Rhamnose		Glycerin	
	Inoculation	Check	Inoculation	Check	Inoculation	Check	Inoculation	Check	Inoculation	Check	Inoculation	Check	Inoculation	Check	Inoculation	Check	Inoculation	Check	Inoculation	Check
1	6.3	6.2	6.9	7.2	6.3	6.1	6.2	6.2	6.3	6.2	6.6	6.8	6.7	6.8	----	----	6.9	6.8	6.6	6.6
2	6.5	6.2	6.7	----	6.6	6.2	6.4	6.1	6.0	6.2	5.8	----	6.1	----	6.3	6.4	7.0	----	6.5	6.2
3	6.6	6.3	6.5	7.2	7.2	----	6.6	----	6.5	6.2	5.4	6.8	5.6	6.8	5.4	6.4	7.2	6.8	6.5	----
5	6.8	----	6.1	----	7.6	----	7.2	----	6.9	----	5.0	----	5.0	----	4.2	----	7.6	----	6.6	----
7	7.2	----	5.7	----	7.8	----	7.6	----	7.2	----	4.6	----	4.6	----	4.2	----	7.7	----	7.2	----
10	7.3	----	5.1	----	7.8	----	7.7	----	7.4	----	4.2	----	4.2	----	4.1	----	7.8	----	7.6	----
15	----	----	4.0	----	----	----	----	----	----	----	4.1	----	4.1	----	4.1	----	7.9	----	----	----
16	7.3	6.1	----	----	7.9	6.2	8.0	6.1	7.4	6.1	----	----	----	----	----	----	----	----	7.6	6.1

These results agree with those from the colorimetric tests except that in the colorimetric tests mannit and glycerin show no acid reaction. However, the colorimetric tests were not continued so long.

TECHNICAL DESCRIPTION

Bacterium Martyniae, n. sp.

A motile rod with rounded ends and polar flagella; single or in pairs, occasionally in chains; average measurements 1.68μ by 0.59μ; no spores; capsules occur; Gram-negative; not acid-fast; growth in nutrient broth moderate, turning light green with age, clouding in bands, with a thin pellicle containing many small rectangular crystals; clouding in Cohn's solution moderate with long triangular crystals growing to three-fourths of an inch in length; clouding moderate in Uschinsky's solution with a heavy pellicle; clouding light in Fermi's solution with a heavy pellicle; beef peptone agar colonies round, smooth, glistening, raised, later becoming umbonate, and turning medium green; gelatin liquefied; blood serum cleared and slightly liquefied; optimum temperature about 25° C.; maximum 37°, minimum 1.5°, thermal death point 49°; indol not produced; H_2S produced; aerobic; little if any diastasic action; soft acid curd formed in milk in 2 days; peptonization completed in 14 days; reduction of litmus beginning in 5 days and completed in 8 to 14 days; methylene blue reduced in 4 to 5 days; nitrates promptly reduced; acid without gas produced with sucrose, dextrose, galactose, arabinose; pathogenic on *Martynia louisiana* (= *M. proboscidea*), producing spots on leaves, and sometimes involving the entire plant.

CONTROL

Diseased leaves and plants should be destroyed as soon as lesions appear, and only seed from healthy pods should be used for planting when Martynia is grown for commercial purposes.

SUMMARY

Plants of *Martynia louisiana* (= *M. proboscidea*) showing bacterial lesions on the leaves were collected from beet fields in Kansas. Isolations from these lesions gave pure cultures of a white bacterium which readily reproduced the disease on healthy plants sprayed with the organism. When severe the disease may infect the fruits and even destroy the entire plant.

The name *Bacterium martyniae* is given to the causal organism. As a means of control, sanitary measures are recommended.

The investigation here presented indicates that *Bacterium martyniae* does not infect sugar beets, young cucumber plants, or the trumpet creeper.

RELATION OF SHEEP TO CLIMATE [1]

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INTRODUCTION

For many years sheep breeders have contended that breeding, feeding, and management are the chief factors involved in the further selection and development of the various breeds of sheep. But evidence from literature, together with the records of the university flock of the University of Illinois (at Urbana, Ill.), indicates that climatic conditions should also be considered.[3] The data here presented seem to support the following: (*a*) Sheep are limited to certain climatic conditions; (*b*) some breeds are better suited to certain climates than to others; (*c*) sheep are especially sensitive in lambing time and rutting seasons; (*d*) rutting season comes with falling temperature and varies from year to year; (*e*) high temperature with high humidity is detrimental to the growth of lambs; (*f*) some years are more favorable than others for lambing, growth of lambs, rutting season, and gestation period; (*g*) housing has extended the limits of sheep production; (*h*) some shelter types are more desirable than others. The climatic diagram methods used here were applied to tropical crops by Taylor (*25*).[4] The writer is not aware of a previous application of this method to a critical period by months and to good and bad years.

WILD SHEEP

The ancestry of domestic sheep is not definitely known. It is most probable that the Mouflon, or European wild sheep, and one of the races of Asiatic urial (*Ovis cycloceros*) have formed the chief parent stocks (Lydekker, *17*, *p. 27*). The Mouflon (*Ovis musimon*) is still found in Corsica and Sardinia. That at one time it had a wider distribution is evidenced by the remains of wild sheep in the superficial deposits of various parts of south Europe. The breeding or rutting season occurs in December and January. The gestation period is about 145 days, and the lambs (generally two) are born in April or May. The wild Mouflon interbreed with the domestic sheep and the offspring are fertile. The body covering of hair has to a minor degree the serrated scales which give to wool its felting property. The cry of the adult is a bleat very similar to that of domestic breeds (*17*, *18*, *19*). Sheep in the wild state are essentially mountain animals. They shun forests, live in the open, and feed upon the mountain grasses. The climatic conditions in such regions as the higher parts of Sardinia and Sicily are probably most suitable for sheep. The days are hot, the nights cool, the winters mild, with more or less rainfall; and the summers are dry (*2*). The sheep avoid the heat in the valleys by moving up the mountains, where it is cooler and where the grazing is less affected by the dry weather.

PRESENT DISTRIBUTION OF DOMESTIC SHEEP

Sheep are not uniformly distributed. There is dense sheep population in some localities and none in others (*6*). These areas of dense population have similar climates. The ideal climates here represented for sheep (fig. 1) are based upon these dense centers. The radical way in which such climates differ from others is emphasized by comparison with Taylor's 1919 diagrams. Each locality probably, though not necessarily, has the breeds which

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[2] The writer desires to express his appreciation to Dean W. C. Coffey, of the University of Minnesota, for suggesting the problem. He is indebted to Prof. H. P. Rusk and to W. G. Kammlade for many courtesies extended during the course of the investigation, especially in the securing of equipment and in giving access to the records of the university flock. The assistance from the graduate school research funds for the purchase of hygrothermographs to carry on a part of the work was invaluable. Thanks are especially due to Dr. V. E. Shelford for his encouragement and for suggesting and supervising the work with the graphic methods employed.

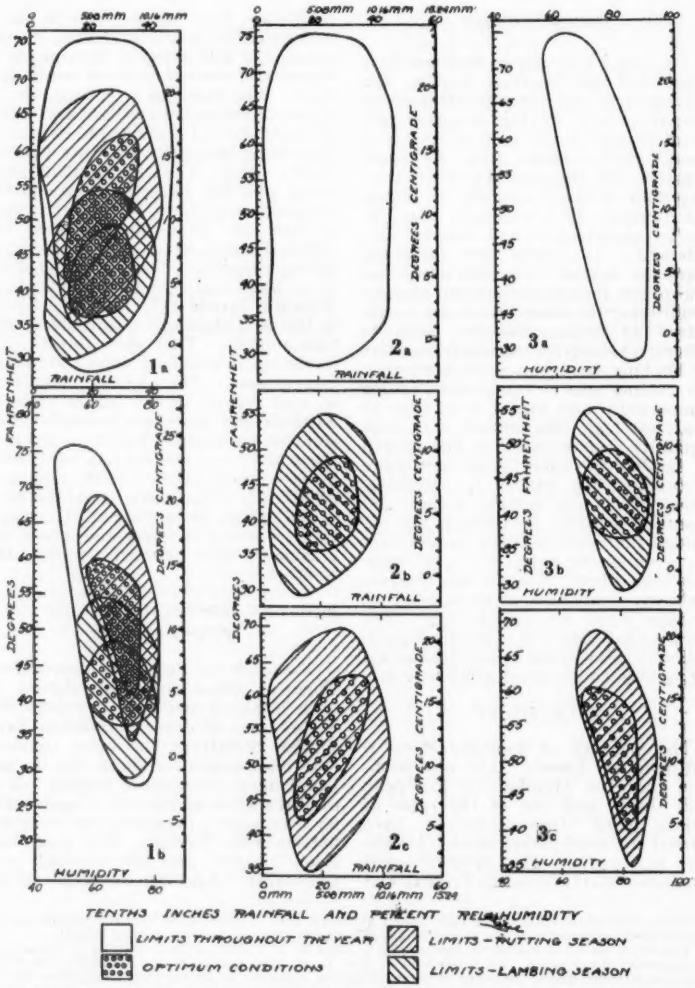
[3] A summary of the more important literature reviewed is contained in a manuscript in the library of the University of Illinois (master's thesis), where important sections are quoted from the original sources and full citations given.

[4] Reference is made by number (italic) to "Literature cited," p. 500.

thrive best, and best meet the needs of the people, as determined by time and varieties tried. Some breeds are suited to fertile valleys with damp climate and swampy soil; others are adapted to moorlands and mountains. Professor Wallace, of Edinburgh University, makes the following statement:

> When local conditions of climate, soil, management, and markets are suitable, the breed is likely to enjoy a fixity of tenure (28).

In the new countries it was difficult to determine which sections were best suited to sheep and what breeds to select (*7*, *21*, *23*). Some were entirely unsuited; others proved suitable to certain breeds only. At one time in the Eastern States the fine-wool sheep were crossed with the large, long-wool sheep, but the resulting cross was not very popular. The wool was too coarse, the carcass too large, and the large sheep, when fat, could not endure



FIGS. 1 to 3.—1a and 1b, composite hythergraph and climograph of dense centers of sheep population in the world; 2b and 2c, composite hythergraph (temperature-rainfall) for critical periods; 3b and 3c, composite climograph (temperature-humidity) for critical periods

hot summers. The many loose-wool sheep resulting from such crosses could not endure cold winter rains. The Down breeds were next used to replace the long-wool sheep, and the resulting crosses were more satisfactory (*27, p. 113*).

The sheep population has not yet reached large numbers in the Corn Belt, probably because of the limited pasturage, and because climatic conditions are unsuitable, as the summers are hot with frequently a rather high humidity. The Southern States have never had large numbers of sheep, and except in Kentucky and Tennessee the sheep are rather scattered. In general, they are small and angular, with a light fleece of poor quality. The fleeces of sheep introduced into these States deteriorate, and the mutton breeds lose their plumpness, evidently because of the need of covering and protection. R. Lydekker (*17, p. 24*) states—

> many tropical breeds of domesticated sheep retain a hairy coat comparable to that of wild sheep, but in other breeds, especially those inhabiting temperate and cold climates, this is more or less completely replaced by wool, except on the face, ears, and legs, where the hair is generally retained,

McKee (*19*) remarks that "sheep are not as good milkers in hot as in cold climates."

The sheepmen in the Western States maintain their flocks in the valleys during the winter and lambing season; then they move the flocks up the mountains, where they remain until fall. Occasionally heavy losses are incurred during these seasons because of the cold and deep snows. A flock of 100 sheep shipped to the Kadiak region of Alaska did fairly well during the summer on the luxuriant growth of grasses, although many suffered from foot rot. When closely confined for protection in winter, their fleeces dropped off in large patches, and emaciation followed (*3, p. 17*).

The sheep introduced into Colombia were the ordinary "Churro," or common Spanish breed. They flourish in the highlands but not in the hot valleys and plains. Unless the lambs are shorn early, the wool becomes loose, falls off in large patches, and is replaced by shining, close-lying hair similar to that of goats. This hair is never replaced by wool (*17, 247–250*). According to Griffith Taylor (*26*), "sheep [in Australia] occupy the warm inland drier belt and the cattle the wetter coastal regions."

On the north island in New Zealand, the Romney Marsh and the Lincoln breeds are found in large numbers, whereas the Merino is found only on the south island, where drier conditions prevail. The Romney Marsh breed comes from the county of Kent, in southeastern England, where the land is low and marshy. They are practically free from foot rot and can withstand cold wet weather. In New Zealand the conditions for the Romney Marsh are more favorable than in Kent, as evidenced by improved quality and length of the fleece. The Lincoln is quite similar to the Romney Marsh, although developed under somewhat different climatic conditions. In regard to moving sheep from one locality to another, Professor Wallace (*28*) states:

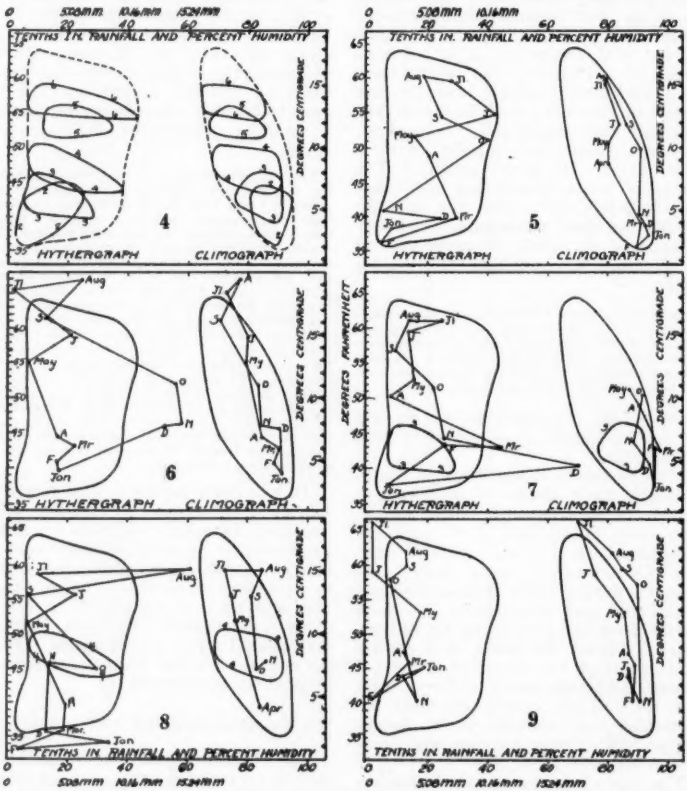
> There is an invariable rule that brooks no breaking that to thrive they must go from a poorer to a richer soil and more favorable climatic surroundings. For example, they must go from a humid into a drier district. It has been claimed that Oxford sheep coming from the cold, heavy clay of Oxfordshire and there subjected to the fogs and low temperatures do better in the drier and warmer localities. The long-wool and down breeds, if put on the mountain lands where the Cheviots, Blackfaces, and Welsh sheep thrive well, could not survive long enough to enable them to make the change. Lincolns, bred in Shropshire, do not attain the great size they do in their home country. The Kerry Hill (a Welsh breed), if removed to places where the soil is deep and the climate damp, get old and crocky or broken down in appearance and slack in their wool. It is claimed that the long wools thrive best on a light, dry soil, on account of the wool not getting so clogged in wet weather.

It has been assumed that sections having a dense sheep population have favorable climatic conditions for sheep production. Such dense world centers exist in South America (Uruguay and northeastern Argentina), South Africa (Basutoland), Australia (New South Wales), New Zealand, Great Britain, European Turkey, and Bulgaria. Russia and the United States also have a large sheep population (*6*).

COMPARISON OF CLIMATES

The climatic factors here considered are temperature, rainfall, and humidity. Comparisons were made by the use of the hythergraph (temperature-rainfall) (*13, 14, 15, 24, 25*) and the climograph (temperature-humidity) (*1, 22*). Graphs were made from the mean monthly temperature, rainfall, and humidity records for various meteorological stations located in sheep-growing sections. Each point on the hythergraph represents the mean of the temperature and the rainfall for a particular month. The points for each month in the year were plotted and then connected. For the climograph the mean monthly temperature and humidity were used. By this method

two factors and the months or critical periods can be shown at one time. In these graphs the temperatures are represented on the vertical scale to the left, and the tenths of inches of rainfall and the per cent of relative humidity on the horizontal scale at the top. (For a more detailed method of construction, see Taylor (*25*)). When possible, records from several stations with averages over as long a period of years as possible were selected for each section.



FIGS. 4 to 9.—4, limits for February, March, April, May, and June of a good sheep year in England; 5, good year, Reading, England, 1909; 6, bad year, Hastings, England, 1911, due to high temperature and humidity during July and August; 7, bad year, Reading, England, 1914, due to wet March and December; 8, bad year, Rounton, England, 1917, due to cold spring period of critical months; 9, bad year, Croyden, England, 1921, due to lack of rain, high temperature, and humidity in summer

These graphs, representing the mean monthly temperature, rainfall, and humidity of the important (densest) sheep centers in the world, were made into a composite graph, made by tracing one graph on top of another and by connecting all the outer points (figs. 1a, 2a, 3a, and 1b). Graphs can thus be made of any section to compare with the composite.

A comparison of the graphs of the important sheep countries shows similarity in rainfall, temperature, and humidity (*2*, *5*, *10*, *11*, *12*, *20*, *29*). In general, the winters are mild and the summers cool, with a moderate amount of rainfall, sufficient to afford a good growth of forage; and the humidity is considerably lower in summer than in winter.

On the composite graph the rutting and lambing seasons have been plotted because sheep seem sensitive during these periods. The lambing season, which extends over three or four months, was obtained for each country and then plotted. The middle month (or months)

was taken as a mean because the bulk of the lambs are born at that time (figs. 1a, 1b, 2b, 3b). The rutting season was determined from the lambing season by counting back five months. The mean of the rutting season corresponds to the mean of the lambing season (figs. 1a, 1b, 2c, 3c).

The lambing and rutting seasons fall within comparatively narrow limits of temperature, rainfall, and humidity, and within narrower limits than does the composite graph for the whole year.

The graphs made from sections which have less dense population than those from which the composite was made show some variations—the climate may be hotter in summer, colder in winter; it may have more or less rainfall, a higher humidity, or a combination of these factors. The lambing or rutting seasons may not fall within the limits of those in the composite. For example, a hythergraph of Cedar Lake, King County, Wash. (fig. 14), for 1919 shows an excessive rainfall in the winter with 21½ inches in January, and a dry summer with 0.5 inch rainfall in July. There were no sheep recorded in this county, as the rainfall is too great in winter and too little in summer. Yet the temperature conditions are favorable, for the range is no greater than in some of the best sheep countries.

CRITICAL PERIOD

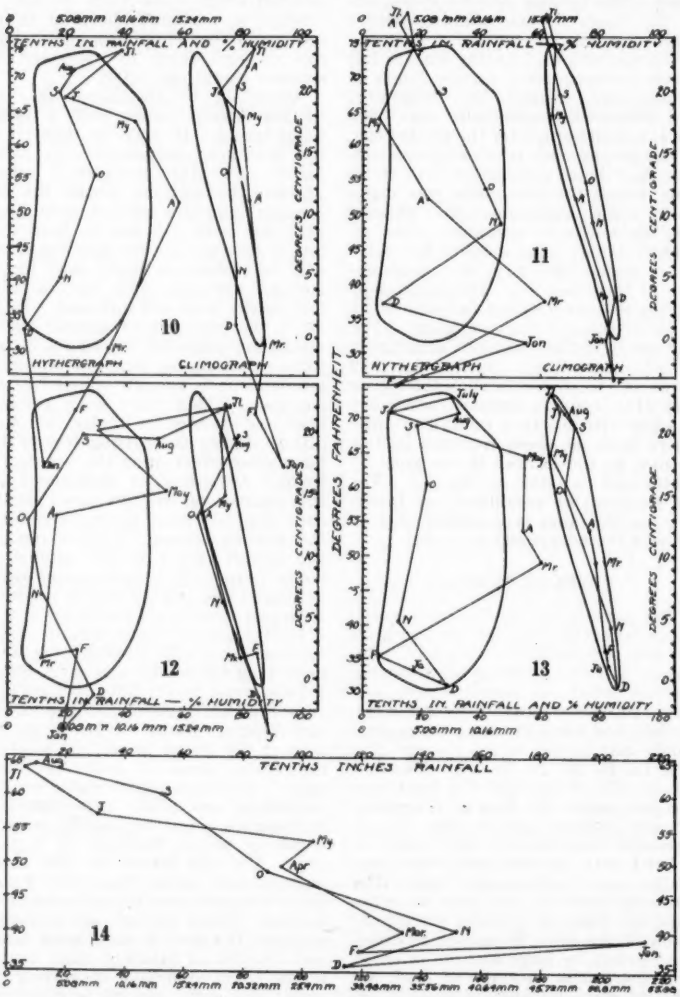
The periods in the life of a sheep during which it is more easily and more seriously affected by unfavorable conditions are the lambing and rutting seasons, which, as stated above, fall within narrower limits of temperature, rainfall, and humidity than the general conditions under which sheep live (figs. 1a, 1b, 2b, 2c, 3b, 3c). The first few months of the life of a lamb and to a less degree the time of pregnancy are also critical periods (fig. 4). A successful lambing season is often interfered with by cold, wet, or snowy weather, or by a shortage of feed. The new-born lamb is not able to withstand too rigorous climatic conditions. Likewise, the ewes, if exposed to excessive rainfall, or large amonts of snow, or if there is a shortage of feed, are liable to come to lambing time in unthrifty condition and give birth to weak lambs. According to Hammond (*8*), if conditions are extremely unfavorable, atrophy of the fetus, due to undernutrition, may occur. (This is much less frequent in sheep than in swine.)

The growth of the lamb during the first few months is readily retarded by hot, humid weather or by cold, wet weather. The extremely hot, humid weather also causes many lambs to lose weight (figs. 15 and 16). The late-born lambs are more severely affected than those born earlier. A breeder of purebred sheep in Illinois has found that clipping lambs in May has enabled them to withstand the summer conditions better.

According to Hammond (*8*), the rutting season comes with a falling temperature. It may be delayed by hot weather, particularly by warm nights (*4*). This becomes a serious problem in sections where the late summers and falls are hot, as it means that the ewes will not be bred until late in the fall, and the lambing season will be correspondingly late in the spring. Sections which have a warm fall usually have hot summers. In the West the ewes found unbred in the spring are gathered together and sent to the mountains, where a decreasing temperature is brought about by high elevation. Then they soon come in heat, and are bred for fall lambs. Conditions during the rutting season may have some effect upon the per cent of twins. According to Hammond (*8*), the number of ova produced by the ewes depends upon their condition at the rutting season. This is possibly the largest factor in the number of lambs born. (It is not true of swine, as many more ova are usually produced than can ever reach maturity.)

In Tennessee, where climatic conditions are generally favorable for lambing during the winter and early spring, the sheep men find it difficult to get their ewes bred early enough in the fall for early spring lambs. If they do not market the lambs before hot weather, the lambs cease to make profitable gains. In Illinois, unfavorable weather conditions are often experienced at lambing time, but can usually be overcome by proper methods of housing (*20*). Yet the lambs do not thrive quite so well during long spells of cold, rainy weather, even though adequately housed. When winters are extremely cold and the snow is deep, weak lambs and trouble at lambing time usually follow. The difficulty of getting the ewes bred early enough is quite frequent. The year 1915 was an exception, as the summer was unusually cool (fig. 12). Ewes came in heat during all the summer months, and the first lambs came in November. The summer months are generally very trying on sheep, and the growth of lambs, particularly those born late in the spring, is retarded. In England it

appears that unfavorable conditions are most likely to occur during the lambing season and in the winter months. The cold, wet weather is very trying to the ewes and lambs; and frequently a cold, wet fall and winter are followed by a small fall of lambs (*16*).



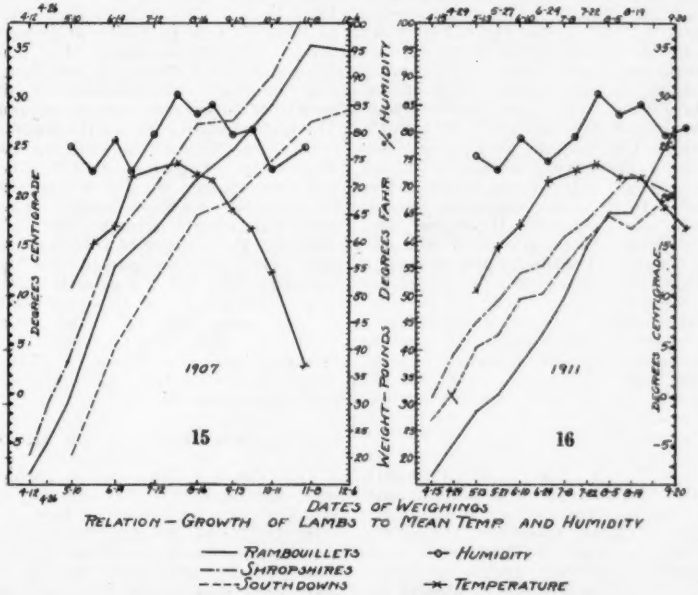
FIGS. 10 to 14.—10, bad year, Urbana, Ill., 1912, due to high summer humidities; 11, fair year, Urbana, Ill., 1913, due to low summer humidities; 12, bad year, Urbana, Ill., 1915, due to high humidity and rainfall; 13, good sheep year, Urbana, Ill., June, 1920; 14, hythergraph, 1919, Cedar Lake, King County, Wash.

GOOD AND BAD SHEEP YEARS

Since some years seem to be more favorable for sheep than others, a study was made of a number of years in south-central England and at Urbana, Ill. (*20*). Hammond (*8*) found the season to have considerable influence on the growth of sheep, particularly during their first year. He attributed most of this to rainfall, which he correlated with the weight at the end of the first nine months of the life of the lamb.

The years 1909 to 1921 (1912 no data) in England were studied, and the infor-

mation regarding the effect of each year upon the sheep was obtained (*16*). The years most favorable and unfavorable for the Southdown and Hampshire breeds were selected. Seasons unfavorable for these breeds were unfavorable for others in most districts in England. A number of meteorological stations were selected in south-central England, the native home of these breeds and the locality in which they are found in greatest numbers. Climographs and hythergraphs were made from the monthly means for the years 1909, 1910, 1911, 1914, 1915, 1917, 1918, 1919, and 1921. From the information pared with a composite containing the limits for April of a good year (fig. 4) it falls far below (fig. 8). A composite was made for the five months only, because they fall within narrower limits than some other months in the year, and come at more critical periods. The graphs for several years differ considerably, and do not always fall within the limits of a good sheep year for England. One critical month may be bad and others good. The graphs for 1909 at Reading, England, fall within the limits of the composite for a good year. That year was satisfactory for sheep (*16*), and in particular for the



FIGS. 15 and 16.—Growth of lambs at Urbana, Ill., 1907 and 1911

at hand, the best lambing seasons and the most favorable summers, autumns, and winters were selected. From these were made a composite hythergraph and climograph, which represents a good sheep year in England. The unfavorable years can be compared with this good year and the contrast noted (figs. 6, 7, 8, 9). As the critical months of a good year fall within narrow limits, a graph was made for February, March, April, May, and June of the good years (fig. 4). For example, too cold an April of any given year, when compared with the composite hythergraph and climograph, might fall within the limits of a good year; but when com- lambing season (fig. 5). The following selected case serves to illustrate bad years due to unfavorable critical months and to poor food and water supply. The summer of 1911 at Hastings was hot, and July was very dry; the humidity was high in July and August; and October, November, and December were very wet (fig. 6). This was noteworthy because of the small number of twin lambs, the failure of food crops, and the low price of mutton. From Figure 7 it appears that March, 1914, at Reading was too wet. Early lambs were weak and mortality was heavy. December of the same year was exceedingly wet, and ewes lost

weight. April, 1917, at Rounton, England was too cold, as were January, February, and March. There was a difficult lambing season with a small lamb crop, but the later months were better. August was too wet, with over 6 inches of rainfall, but the humidity was not high. In 1921 at Croyden the lambing season was favorable (fig. 9). The summer was particularly dry, accompanied by humidity a little above the limits. Water had to be carted for livestock for six months.

A comparison was made between the good and bad years at Urbana, Ill., from 1906 to 1922 (*20*). (The information obtainable was not so complete as that for England.) The rate of gains made by the lambs was taken as an indication of the effect of the summer conditions upon them. The mean weights of each breed—Southdowns, Shropshires, and Rambouillets—were plotted for the years 1907, 1911, 1913, 1915. The weights were taken each month, and twice a month during some years. The mean temperatures and humidity for two-week periods were also plotted. Hammond has shown the normal growth of lambs to be regular. Figures 15 and 16 show a comparison of the growth or increase in weight with the mean temperature and humidity. The irregularities indicate that a high mean temperature accompanied by high humidity is correlated with the retarded growth of the lambs. In some cases there was a loss in weight for a two to four week period. The graphs presented here (together with others on file at the University of Illinois) show that retarded growth follows high temperature and humidity regardless of the date or the age of the lambs. Curves drawn for 1913 (on file at the University of Illinois) show comparatively uniform growth of lambs (fig. 11). The difficulty in making use of the weights of the university flock grew out of the different dates of lambing, evidently influenced to some extent by the weather condition of the preceding mating season. Mean growth was rendered insignificant by this irregularity, and retardation in growth was noted at critical periods regardless of the age of the lambs.

A mean relative humidity higher than 80 per cent and a mean temperature higher than 70° F. seem to be detrimental. The sheep can stand a rather high mean temperature if the mean humidity is not over 60 or 65 per cent. The rate of growth was more uniform in 1913 than for the other three years plotted. The study has not been carried far enough to give definite limits of temperature and humidity. The Rambouillets, which come from a country where the temperature runs higher than it does in the section from which the Shropshire and Southdowns come, seem to be less affected by unfavorable conditions. The late-born lambs are more seriously affected by unfavorable conditions than the early-born lambs.

A good sheep year at Urbana, Ill., closely approximates the limits of a good sheep year in England, although it has a wider range, being warmer in summer and colder in winter. Figures 10, 11, 12, and 13 are hythergraphs and climographs of the years 1912, 1913, 1915, and from June, 1920, to June, 1921, respectively, at Urbana, compared with the limits of a good sheep year at Urbana. It was found that summers unfavorable for the growth of the lamb are rather common. The high humidity during the summer of 1912 was thus detrimental (fig. 10). During the hot summer of 1913, which was accompanied by a comparatively low humidity, the lambs made a regular and satisfactory growth (fig. 11). Although the summer of 1915 was cool and wet, the comparatively high humidity retarded the growth of the lambs, particularly of those born late in the spring (fig. 12). The period from June, 1920, to June, 1921, was a favorable year for sheep, and falls almost entirely within the limits of a good sheep year (fig. 13).

HOUSING OR SHELTER FOR SHEEP

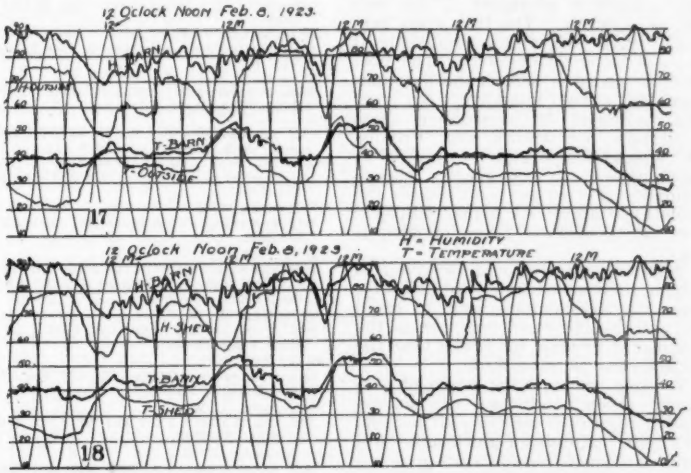
The climatic limits under which sheep can be successfully kept may be extended by providing shelter and extra food. In the important sheep countries the sheep are seldom housed, and depend largely upon grazing for their food supply, although in some sections some additional food is provided during the winter. Shelter or housing makes it possible to raise sheep successfully in sections which have low temperatures, heavy snowfall, or excessive cold winter rains. The types of shelter may vary from sheds, constructed to break the wind and protect them from the cold rains, to warm well-constructed barns. In comparing graphs of these sections with the composite graph for good sheep countries, one should make some corrections for housing and various kinds of shelter. But no data have been found on how much correction is advisable.

The sheep farm at the University of Illinois has two types of building—one a shed which is open to the south,

the other an inclosed barn with a loft above for hay and feed storage. In order to make corrections for temperature and humidity, a hygrothermograph was placed in each building, and one out of doors, making possible a comparison between the shed, barn, and outdoor conditions. These hygrothermographs were placed in the pens where the sheep were kept; the one out of doors was in a shelter about 60 rods south of the buildings. All of the instruments were placed 12 to 15 inches above the ground because this distance is approximately the mean of the center of the body of the sheep when standing and lying down. The records from the three instruments began February 1, 1922, and have been continued to the present time.

As yet there are not sufficient data to make accurate corrections for housing, but a marked difference is indicated by the records (fig. 17). The mean temperature was 3.4° to 10.2° F. higher in the barn than outside. The mean humidity was more variable, in some cases 13.6 per cent higher and others 6.7 per cent lower than outside. The differences between the shed and the outside were very small.

In the barn the temperature and humidity are more uniform with much less marked fluctuations than in the shed or out of doors (fig. 18). The barn is warmer than the shed in cold weather, but does not become warm so quickly on a mild sunny day in winter. The humidity runs higher in the barn, which frequently is steaming when opened in the morning. Some trouble from pneumonia and other respiratory diseases has been experienced with the sheep kept in the barn, but practically none with those kept in the shed. A study of the daily range and the maximum and minimum temperatures and humidities may also show a greater difference between the barn, shed, and outside. On many farms the housing is inadequate, with a resulting detrimental effect upon the ewes, especially where they are closely confined during very cold and snowy weather. After a winter of this kind, the lambs are usually born weak and as a consequence are less thrifty. This is possibly due also to a lack of exercise.



FIGS. 17 and 18.—Comparison of temperature and humidity in barn, shed, and outside, Urbana, Ill., 1922

CONCLUSIONS

The dense centers of sheep population in the world are found within comparatively narrow limits of temperature, rainfall, and humidity. The mean temperature ranges between 28° and 77° F.; the rainfall between 0.3 and 4.5 inches per month; the relative humidity between 55 and 70 per cent at the higher temperatures and 65 and 91 per cent at the lower temperatures. Such conditions afford mild winters, cool summers, and sufficient rainfall to provide good grazing.

Climatic conditions must be favorable, especially during the critica periods of rutting season, pregnancy, lambing, and the growth of the lambs

The lambing period generally comes in the spring, enabling the lamb to get a good start before hot weather sets in. In the spring there is also an abundance of forage for the suckling ewe. The growth of the lamb is retarded by high temperature and humidity, by excessive rainfall and cold weather. The rutting season comes with a falling temperature and possibly a rather wide range between the maximum and minimum temperatures. Unfavorable conditions at the rutting season or during the gestation period tend to produce a small fall of lambs, and often weak lambs. If these critical periods are greatly disturbed, sheep are not likely to be numerous or profitable.

In Illinois, conditions of good sheep years are found to resemble the average conditions prevalent in the best sheep countries. The bad years generally have unfavorable conditions at some of the critical periods. The most serious condition here is the hot summer with a high humidity. No practical methods of overcoming these conditions are known. At Urbana, Ill., a mean temperature of over 70° F. accompanied by a mean relative humidity over 80 per cent, retards the growth of lambs. A higher temperature can be tolerated if the humidity is lower.

More attention should be given to finding breeds best suited to a locality, since the Rambouillets seem to stand the unfavorable conditions during the summer at Urbana, Ill., better than the Southdowns or Shropshires.

In some sections the limits of successful sheep production can be extended by proper methods of housing. The barn maintains a much more uniform and higher temperature and humidity than the shed or out of doors. But conditions in the barn are not entirely satisfactory for the health of sheep.

Sheep thrive best where cool summers and mild winters prevail and where sufficient rainfall is afforded to provide good grazing.

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TOLERANCE AND RESISTANCE TO THE SUGAR CANE MOSAIC[1]

By C. W. EDGERTON, *Plant Pathologist*, and W. G. TAGGART, *Assistant Director*, *Louisiana Agricultural Experiment Station*

A survey of the literature of mosaic diseases discloses a rather general belief among pathologists that (1) a plant once affected with mosaic is always so affected and (2) that with favorable environmental conditions an affected plant shows a gradual deterioration. There are a few recorded exceptions, but the small number of these makes only more prominent the general belief.

Where plants are propagated by seed, a condition of this nature is not particularly important, since apparently but few mosaic diseases are transmitted from one year to the next through seed. But where plants are propagated vegetatively, if this belief is true, the resulting conclusion is serious, for with plants like sugar cane or potatoes which are propagated by cuttings or tubers, a plant once affected must always remain affected, and after a sufficient length of time should show a deterioration that would make it unfit for planting. The only hope in such a situation would seem to be to grow healthy plants in some section where the mosaic did not spread rapidly and thus to keep a supply of healthy plants for propagation purposes. Such a course might be possible with potatoes but it would be impossible with sugar cane in a State like Louisiana, where the requirement for planting is from 3 to 5 tons per acre. If with such a crop the spread of the mosaic is extremely rapid—as it is on sugar cane in Louisiana—a 100 per cent infection must soon be reached, and with no healthy cane for planting we should expect from the general belief that there would be a gradual deterioration and possibly the failure of the crop unless fortunately a resistant variety could be found which might be substituted for the susceptible ones now being grown.

If, on the other hand, we consider conditions as they actually are, we find that in countries like Java, where sugar cane has been infected with mosaic for many years, a decidedly different situation. Instead of the canes being in bad condition we find what are called "tolerant" canes, canes that are affected with the mosaic but are not injured to a great extent. In many of the tropical sugar countries where the mosaic disease has been severe in recent years the Java seedlings are replacing the old standard varieties because of their tolerance to the mosaic. We also occasionally find in other countries the occurrence within a variety of a strain which is markedly resistant or immune. Possibly the Toledo cane which was reported from the Philippines in 1923 is an example of this.[2]

From the experiments and observations on the mosaic disease in Louisiana made during the past several years there have developed a number of things which have a bearing on the general mosaic problem and which also may be important in the control of mosaic diseases on vegetatively propagated plants. These results have a direct bearing on the general statement made in the introductory paragraph of this paper.

Soon after the recognition of the mosaic disease on sugar cane in Louisiana it became evident that conditions were excellent for its rapid spread and that it was useless to try to stamp it out or even to keep it under control. The Louisiana workers realized that the disease would spread rapidly across the State and would soon reach a point of maximum infection. As a matter of fact, the spread has been faster than was even anticipated at that time. Furthermore, experiments to test the possibility of keeping the disease in check by roguing the fields proved futile. Confronted with the idea that a plant once affected is always affected and that plants affected with a mosaic disease show a gradual deterioration, a solution of the problem did not seem remarkably hopeful. However, several lines of investigation were started.

[1] Received for publication May 24, 1924—issued February, 1925.

[2] HIND, R. R. TOLEDO CANE, A MOSAIC-IMMUNE VARIETY. Sugar Cent. and Plant. News. 4:105-107, 110, illus. 1923.

RAPIDITY OF NATURAL INFECTION OF CANE MOSAIC

The first work attempted with the sugar cane mosaic was to test out the different varieties of cane that are grown in Louisiana. It was very soon determined that the common commercial varieties, including D 74, D 95, Purple and Striped, were very susceptible to the mosaic, and that if healthy cane is planted in a badly infected field the crop will show practically 100 per cent infection before the end of the first season. It was also determined that diseased canes of these varieties when planted produced diseased plants the next year. This is in accord with the prevailing opinion in regard to mosaic transmission. In Table I are given the results of a few tests showing the rapid increase of mosaic on some of the susceptible canes when grown in a badly infected field.

TABLE I.—*Rate of increase of mosaic infection on susceptible canes*

Year	Variety	Percentage of mosaic on different dates				
		May	June	July	August	September
1920 [a]	D 74				100.0	
1920 [a]	D 95				100.0	
1921	Palfrey	4.4		56.6	100.0	
1922	do	1.9	36.6	47.0		(b)
1922	Purple	0.0	42.1	63.0		(b)
1922	D 74	1.8	17.8	64.0		(b)

[a] Most of the data for 1920 was lost by fire.

[b] The cane was examined in September, 1922, but since all of the plats showed practically 100 per cent infection counts were not made.

RESISTANCE TO INFECTION

It was noted, however, that cane of another variety known as L 511, acted in a manner different from the common canes. The L 511 is one of the canes developed at the Louisiana Experiment Station. It is very rich in sucrose, but, since the tonnage is usually light, it is not becoming generally planted. Plants of this variety when affected with mosaic show the disease in a form as severe as that of any of the other varieties, but only a moderate percentage of the plants in a field show the presence of the disease. One of the reasons for this has been determined to be the resistance of the plants to infection. Resistance to infection should be kept distinct from the relative severity of the disease after infection occurs.

The L 511 is one of the canes which shows the mosaic on the stalks as well as on the leaves. Healthy stalks are a light green in color but diseased ones have red stripes running in the direction of the main axis. For a number of years stalks lacking these stripes, that is, stalks free of the mosaic, have been selected and planted and the crop watched through the following year. The increase in mosaic on these plats is shown in Table II. In all cases the increase in mosaic has been very slow as compared to that on susceptible cane varieties. This shows a resistance to the infection but has no reference to the severity of the disease following infection. The stalks that became affected showed the disease in as intense a form as do susceptible varieties. To what this resistance to infection is due has not been definitely determined. It may, however, be the result of a better protection of the growing bud. The cluster of leaves at the top of a stalk of L 511 cane is much more compact than that on most varieties, and it is possible that this hinders the insects which carry the mosaic from attacking the young bud.

TABLE II.—*Rate of increase of mosaic infection on L 511 cane*

Year	Percentage of mosaic on different dates					
	May	June	July	August	September	October [a]
1921	2.2		21.5	55.0		43.7
1922	13.6	8.9	19.0		24.0	22.6
1923		9.2	10.3		12.3	9.2

[a] The October counts were made at the time when the cane was being cut for planting and were based on the number of stalks that showed the presence of red stripes. The other counts were based on the quantity of leaf infection apparent on the growing cane.

SELECTING FOR MOSAIC TOLERANCE

The work on the problem of sugar-cane mosaic which is considered by the writers to be the most important is the attempt to obtain more resistant or tolerant strains of our common commercial varieties. It is recognized that it is difficult to change or to improve a plant that is propagated vegetatively, yet there seeemed to be two possibilities worthy of testing. (1) Will sugar-cane varieties which show a moderate susceptibility or a moderate tolerance to mosaic vary enough, possibly by bud variation, to produce some plants or strains which will become more tolerant than the rest? Previous work on vegetatively propagated plants, such as potatoes, has shown that but little can be done in improving a variety, and it seemed very doubtful if any selection would improve the resistance of sugar cane. (2) Will a plant that is affected with mosaic, if it is propagated for a number of years, gradually acquire more resistance or possibly an immunity to the disease? The acquiring of immunity is a common thing in human and animal diseases, but no example of a plant acting in a similar manner has ever been demonstrated. It would seem, however, if a plant could acquire an immunity to any disease it would be to a mosaic.

Experiments to test these two possibilities were begun in the fall of 1920. At planting time selections were made in fields of D 74 and Purple cane in a region that had had for several years a 100 per cent infection of mosaic. Stools showing the least injury from mosaic were selected. No cane showing a total freedom from the disease was used, for healthy cane was not desired. What was wanted was infected cane showing a minimum amount of damage. This cane was planted by the side of unselected cane in a field showing a maximum infection. In the fall of 1921 the best cane from the selected plat was used for planting, and from this in the fall of 1922 the cane showing the least damage from mosaic was again planted. The results for the three years 1921, 1922, and 1923 are now available. Counts of the mosaic were made at frequent intervals and the comparative yields were obtained for the last two years. The results are shown in tabular form.

RESULTS WITH D 74 CANE

On the basal or younger portion of a D 74 leaf the mosaic shows in the rather usual form of somewhat lighter colored stripes or areas, but as the leaves grow out portions of these stripes tend to fade out and appear in the form of light-colored, sometimes nearly white, dots or slightly elongated spots (Pl. 1, A, *a*, *b*). On canes showing least damage from mosaic these spots may be so reduced during the latter part of the growing season that it is difficult to say whether the mosaic symptoms are present (Pl. 1, B, *a*). With this variety canes bearing the smallest and fewest light-colored dots or areas were selected for planting. The results obtained from the selection work with the D 74 variety are given in Table III.

The contrast between the selected and the unselected rows in the field was much more striking than appears in the figures in the table. The selected rows were darker green in color and the canes were not only larger but had stooled more. An examination of the individual plants showed that those in the selected rows did not have the mosaic symptoms in nearly so marked a form as in the unselected. Although all of the plants were known to be affected at the time some of the examinations were made, it was absolutely impossible to be certain that mosaic symptoms were present on some of them. While it is difficult to show sugar-cane mosaic in a black-and-white illustration, an attempt has been made in Plate 1, A and B, to bring out the contrast between the leaves from the selected and the unselected rows. These illustrations were made from photographs taken on September 15, 1922. Plate 1, A, shows the white dotted appearance very common with this variety and also the smaller dots on the leaf from the selected row. In Plate 1, B, which illustrates the more common condition of the leaves in these tests, the white dots from the selected row (*a*) are very few while the mosaic symptoms on the leaves from the unselected rows (*b* and *c*) are very obvious. These leaves do not show an exaggerated comparison, but are somewhere near the average.

How much of the 32 and 34 per cent increases in yield shown in 1922 and 1923 is due to the reduced effect of the mosaic is a question. It may be claimed that other factors of health and vigor were unconsciously considered when the selections were made each year. For the 1923 test no stool was used unless it contained at least four good stalks. There is little doubt, however, that a portion of the increased yield was due to the less serious damage caused by the mosaic.

TABLE III.—*Results obtained from selecting D 74 cane for tolerance to mosaic*

Year	Selection	Percentage of mosaic during the season				Yield in tons per acre
		May [a]	June	July	September	
1921 [b]	Unselected	40.0		63.0		
	Selected	30.0		28.0		
1922	Unselected	88.7	100.0	100.0	100.0	10.58
	Selected	58.0	94.1	90.0+	100.0	14.60
1923	Unselected		96.8	100.0	100.0	8.85
	Selected		85.9	100.0	(c)	11.90

[a] The percentages for May are too low on account of the very many small plants on which the mosaic could not be estimated satisfactorily.

[b] For 1921 the figures show only the percentages of pronounced mosaic. Most of the data for that year were lost by fire.

[c] A satisfactory count could not be made in September, 1923, since so many of the selected plants showed the disease in so mild a form.

TABLE IV.—*Results obtained from selecting Purple cane for tolerance to mosaic*

Year	Selection	Percentage of mosaic found during the season				Yield in tons per acre
		May [a]	June	July	September	
1921 [b]	Unselected	45.0		95.0		
	Selected	25.0		52.0		
1922	Unselected	93.6	100.0	100.0	100.0	17.37
	Selected	69.1	75.9	90.0+	(c)	19.89
1923	Unselected		100.0	100.0	100.0	18.5
	Selected		82.2	100.0	(d)	19.7

[a] The percentages for May are too low on account of the very many small plants on which the quantity of mosaic could not be estimated satisfactorily.

[b] For 1921 the figures show only the percentages of pronounced mosaic. Most of the data for that year were lost by fire.

[c] A satisfactory count could not be made in September, 1922, in the selected plat since less than 10 per cent of the plants showed clear-cut mosaic symptoms.

[d] A satisfactory count was impossible in September, 1923, in the selected plat on account of the poorly developed mosaic symptoms.

RESULTS WITH PURPLE CANE

On the Purple cane the mosaic appears in the ordinary light-colored stripes which are characteristic of this disease on many varieties. On severely affected plants these stripes constitute more than 50 per cent of the leaf surface and, consequently, the plants have a yellowish appearance rather than the normal dark green. Selections were made in the same manner as with the D 74 variety. The results are given in Table IV.

The contrast between the selected and the unselected row in the Purple plats was more striking than in the D 74 plats. The selected rows were dark green in color and had the appearance from a little distance of being perfectly healthy. Since these plants were also taller, there was a striking difference in the appearance of the different rows. At some seasons it was impossible to make mosaic counts on the selected rows because of the poorly developed mosaic symptoms. On a great many of the plants even at the

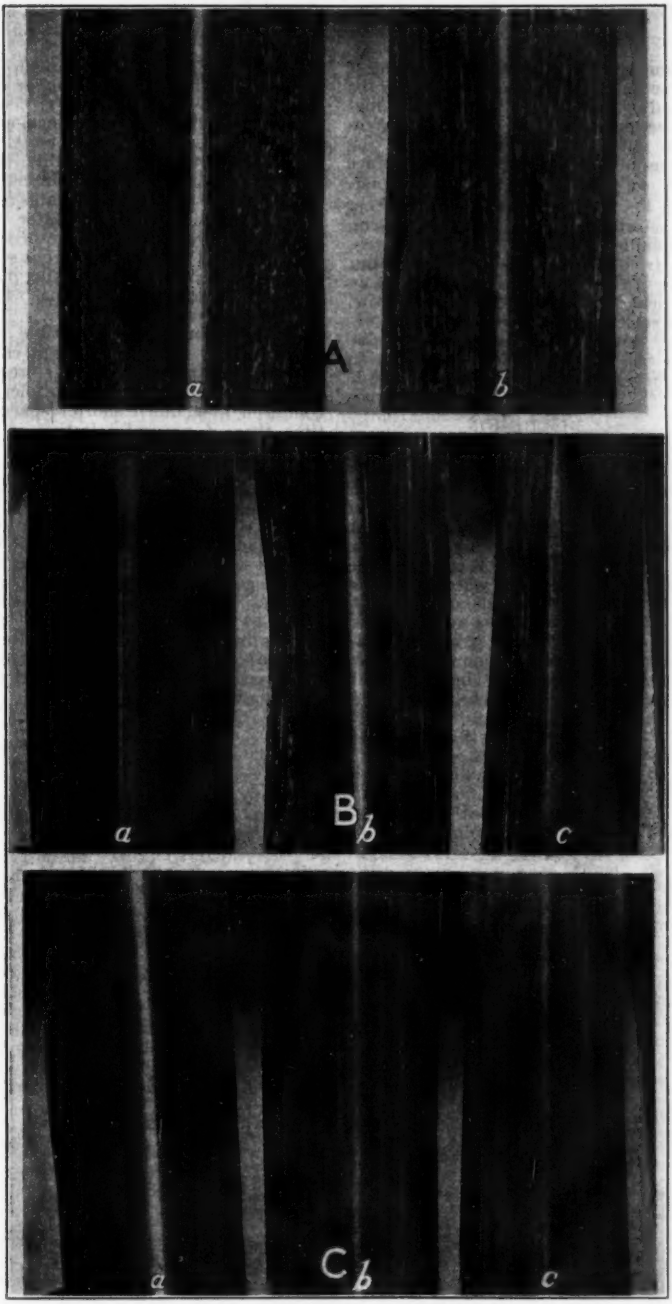
EXPLANATORY LEGEND FOR PLATE 1

Mosaic appearance of sugar cane leaves from plats planted with selected and unselected cane:

A.—The white dot effect of the disease on D 74 cane; *a* from the selected cane, *b* from the unselected.

B.—Average condition of D 74 leaves from selected and unselected plats; *a* from the selected cane, *b* and *c* from the unselected.

C.—Average condition of the leaves of the Purple variety from selected and unselected plats; *a* from the selected cane, *b* and *c* from the unselected.



Resistance to the Sugar Cane Mosaic PLATE I

(For explanatory legend see p. 594)

season when the symptoms were most pronounced the mosaic could only be found by examining the basal or younger portion of the leaves. As the leaves grew out the light-colored stripes disappeared and the whole leaf surface took on a normal dark green color.

In Plate 1, C, are shown leaves from the selected and the unselected rows on September 15, 1922. All the leaves are from mosaic-affected plants, but none of the symptoms are apparent on the leaf taken from the selected row. The selected rows also showed a somewhat larger yield and it is probable that this increase was largely due to the decreased damage from mosaic.

DISCUSSION

The tests with the D 74 and the Purple canes have demonstrated that in fields with a 100 per cent infection plants can be selected that show a marked tolerance to the mosaic. How this has come about is as yet not clear. It is uncertain whether this is due to slight variations of the host with the variants more resistant, or whether the plant is gradually acquiring an immunity somewhat similar to acquired immunity in man and animals. As far as usefulness in the control of the mosaic is concerned, it is immaterial which explanation is correct.

If resistant strains are developing in nature, it is only a matter of time until the low-yielding, highly susceptible plants will be eliminated. Each year the percentage of stalks from tolerant canes would be greater and naturally a greater percentage of such would be used for seed. There is some evidence that this may be taking place in some sections of Louisiana where the oldest infections occur.

By discarding the susceptible and selecting only the most tolerant canes for seed, it is possible that our present varieties can be developed to a tolerant condition in a much shorter space of time than if natural selection is allowed to take its course.

This paper is not an argument for allowing the disease to take its natural course in all sugar countries. In those regions where the disease can be kept under control by roguing or by the use of clean seed it would be folly to let the mosaic go and try to select resistant varieties, but in places like Louisiana where no clean seed is available and roguing has been a failure this offers a possible solution of the problem, at least until satisfactory resistant varieties become available.

FURTHER STUDIES ON THE RELATION OF ONION SCALE PIGMENTATION TO DISEASE RESISTANCE[1]

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STATEMENT OF THE PROBLEM

In earlier studies of the nature of parasitism and resistance associated with the smudge disease (*Colletotrichum circinans* [Berk.] Vogl.) of onion bulbs (*3*, *4*)[2] it has been shown that the host tissue contains certain substances which are toxic to the invading organism. These substances fall into two classes, (a) the volatile oils and certain associated nonvolatile compounds in the cell sap (*6*) and (b) the toxins associated with scale pigmentation. The volatile substances which arise from the succulent tissue, especially upon wounding, are readily taken up by liquid suspensions of conidia and when present in sufficient concentration completely inhibit germination and retard growth. No marked consistent difference in the amount of these volatile toxins given off by different varieties of onion has been noted, and the conspicuous resistance of colored-bulb varieties as compared with the white types is not to be explained on this basis. The volatile substances, while generally toxic to fungi, apparently are not so effective upon rapid bulb-decaying organisms, such as *Botrytis allii* and *Fusarium cepae*, as upon milder parasites, such as *Colletotrichum circinans*. It has been suggested therefore that these toxins in the juice of succulent onion scales may have a rôle in restricting the advance of certain invading organisms (*6*).

The second group of toxic substances was found in the water extract from the dry outer colored-bulb scales from which the volatile substances had disappeared (*4*). Spores placed in extracts of sufficient concentration were either entirely prevented from germinating or, if germination started, the process was so modified as to cause the exudation of naked cytoplasm from the tip of the young germ tube, thus inactivating the organism. As the concentration of the extract was reduced, the toxic effects were proportionately decreased until eventually normal germination and growth occurred. In contrast to this, extracts from white scales showed little or no toxicity and supported good germination and growth. It appeared therefore that the natural resistance of colored onion bulbs to the smudge organism is based upon the toxic substances associated with the pigmentation of scales. It is presumed that in nature amounts of these toxins sufficient to inactivate the fungus (if present) dissolve into drops of meteoric or soil water which come in contact with colored scales. The outer colored scales therefore serve as a barrier to invasion by the parasite.

In the course of this investigation it became of interest to extend the same line of experimentation to a number of other fungi, especially those which are parasitic upon onion bulbs. The relation of onion oils to a number of organisms has been reported (*6*). The present paper deals with the relation of the pigment extracts to practically the same groups of fungi, namely, *Fusarium cepae* Hanzawa, *Fusarium* sp. No. 45 (an onion bulb-rotting organism), *Fusarium lycopersici* Sacc. (tomato wilt organism), and *Fusarium graminearum* Schwabe (*Gibberella saubinetii* (Mont.) Sacc.) (wheat scab organism); onion neckrot fungi, *Botrytis allii* Munn, *Botrytis* sp. No. 110, and *Botrytis* sp. No. 108a; *Aspergillus* sp. No. 4660[3] and *Aspergillus niger* van Tiegh.; and an unidentified species of

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[2] Reference is made by number (italic) to "Literature cited," p. 514.

[3] As noted in another article (*6*), this species will readily decay onion bulbs. It is a yellow-spored form which is being studied by Dr. Charles Thom, Bureau of Chemistry, U. S. Department of Agriculture, and for the present is referred to by his number, 4660.

Helminthosporium which occurs on the dry outer scales of white onions. Varietal resistance to these groups is not uniform. No conspicuous resistance to the Fusaria has been observed (*7*). In the case of neckrot, however, the disease is more prevalent on the white than on colored varieties. *Aspergillus niger* is common on both colored and white varieties. *Helminthosporium* sp. has been found only on white varieties. The data secured for each group and their possible bearing upon paraistism and resistance will be considered separately.

METHODS OF EXPERIMENTATION

The method previously described was employed (*4*). Spores of the organism were suspended in sterile water, and equivalent drops of the suspension were transferred to glass slides in moist chambers. Uniformly white or colored, dry, outer scales were secured from White, Red, and Yellow Globe onions. These were cut into 2 mm. squares. Rapid diffusion of toxin into the liquid followed the addition of these fragments to the drops. To gain a gradation of concentration, one, two, and four fragments, respectively, were added to a series of three drops for each type of onion scale. This method, while obviously subject to some experimental error, gave the general comparison which was desired. Control drops were included in each series.

As will be shown presently, the toxicity of the pigmented extracts was quite general and the toxic effects were of the same general type as those noted with the smudge organism (*4*).

The effect on germination was exhibited either as complete inhibition or by the exudation of cytoplasm from the tip of the germ tube, usually shortly after the germ tube had formed. The latter reaction was not constant, however. It would seem that a very delicate balance in the concentration of the toxin is necessary to bring about the phenomenon. Too high a concentration of toxin evidently completely inhibits germination; too low a concentration allows the more normal hyphal development. At a certain point initiation of growth is permitted, but this is soon checked in such a way as to bring about the exudation of cytoplasm. The nature of this phenomenon is not entirely explained. It is suggested that the toxin retards or stops the wall-forming process at the tip of the germ tube, while imbibition of liquid by the spore protoplast continues. This leads to "rupturing" of the cell membrane at the weakest point followed by exudation of the cytoplasm. In certain cases the presence of a membrane around the exuding cytoplasm is suggested, but more often no such confining membrane can be distinguished.

When hyphal development does occur, the rate of growth is influenced by the concentration of the toxin. The most reliable criterion for comparison was found to be the percentage of normally germinating spores and the rate of germ-tube growth of the spores which germinated. These were estimated after a given interval by counting 100 or more spores and measuring the length of 40 or more germ tubes in each drop. As previously noted with *Colletotrichum circinans*, decrease in concentration of the toxin caused all the organisms studied to return gradually to normal germination and growth. Consequently the effects were not the result of lack of food but were due to the presence of inhibitory substances. In this report it has been considered sufficient to give the data secured from the exposure of the various organisms to the highest concentration of the toxins, that is, where four fragments of scale tissue were added to the drop.

RELATION OF PIGMENT TO DEVELOPMENT OF CERTAIN SPECIES OF FUSARIUM

Two strains of Fusarium which had been proved to be pathogenic to onion bulbs were used. One of these was isolated from bulbs collected in Illinois and agrees with the description of *Fusarium cepae* Hanzawa. Further studies of this disease are reported in another paper (*7*). The second strain was isolated from a decaying onion at Valencia, Spain. Since it has not as yet been identified with any previously described species, it is referred to in this paper as *Fusarium* sp. No. 45. Two species of the genus, nonpathogenic to onion, were included—one a vascular parasite of tomato, *Fusarium lycopersici* Sacc., and the other a parenchymatous invader of wheat and corn, *Fusarium graminearum* Schwabe (*Gibberella saubinetii* (Mont.) Sacc.). Tests with *Colletotrichum circinans* were included for comparison.

From the data given in Table I it will be seen that the addition of white scale tissue to the spore suspension did not materially affect the percentage of spore germination of any of the organisms studied, with the exception of the tomato Fusarium, in which case there was some reduction. The addi-

TABLE I.—*The effect of water extracts from dry outer scales of White, Yellow, and Red Globe varieties of onion on the germination and growth of several species of Fusarium as compared with Colletotrichum circinans*

Organism	Experiment No.	Germination in—				Length of germ tubes in—			
		Control	White scale extract	Yellow scale extract	Red scale extract	Control	White scale extract	Yellow scale extract	Red scale extract
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Microns*	*Microns*	*Microns*	*Microns*
Colletotrichum circinans	1	99	80	0	2	414	224	0	0
Fusarium cepae	1	95	95	0					
	2	87	98	8	6	582	368	134	17
F. sp. 45	1	100	95	23					
	2	50	50	1	0				
F. lycopersici	1	99	56	0	1	96	36	0	
F. graminearum	1	95	95	1	0				

tion of dry, outer, yellow or red scale tissue, however, resulted in a general reduction of germination or incomplete inhibition. The effects are quite as marked as earlier noted for *Colletotrichum circinans*. The growth of the germ tubes is somewhat reduced by the addition of white tissue in the case of both *Fusarium cepae* and *Colletotrichum circinans*. With *Fusarium cepae*, where some germination occurred in colored scale extract, marked retardation of growth is evident. As found with *Colletotrichum circinans*, no striking difference in the comparative toxicity of extracts from red and yellow scales was noted.

The data show that onion Fusaria are distinctly inhibited when subjected in this way to water extracts from dry, outer, colored scales. From the tests with two other species of Fusarium it may be expected that the toxins are equally effective upon forms other than those parasitic on onion bulbs. Should these organisms normally invade the bulb through the outer scales we might expect a difference between white and colored varieties in susceptibility, as generally noted for onion smudge. Since, however, the onion Fusaria normally invade through wounds or possibly through natural openings in the stem plate (7), the possibility of the scale toxins coming into immediate contact with the invading parasite is very slight. Consequently, little or no evidence of resistance to Fusarium bulb rot can be expected among colored varieties, and this supposition is confirmed by the writers' studies so far. In another paper (7) a field experiment is reported wherein plantings of several varieties of onion, including the three color types, were artificially inoculated with *Fusarium cepae*. Infection results at the end of the season were as follows:

	Percentage of fusarium bulb rot
Variety:	
Red Globe	12
Yellow Globe	8
White Globe	18
White Portugal	8

There is therefore no marked indication of varietal resistance between the colored and white varieties in this instance. Further study on this point is needed.

RELATION OF PIGMENT TO DEVELOPMENT OF CERTAIN SPECIES OF ASPERGILLUS AND HELMINTHOSPORIUM

Aspergillus niger is found commonly after harvest on the outer scales of onion bulbs, where it develops largely as a saprophyte under sufficiently moist conditions. Aggressive invasion of the fleshy scales is seldom if ever to be found. Although perhaps somewhat more common and conspicuous on white varieties, the colored forms are often affected as well. *Aspergillus* sp. 4660 was isolated from decaying garlic bulbs and by inoculation experiments has been shown to be an active parasite of the succulent scales of onion.

The data in Table II show that neither of these forms is inhibited by outer white scale extract. In the case of *Aspergillus* sp. 4660, germination was always better in the white scale extract than in the control; as a rule it was either decidedly retarded or entirely inhibited in the colored scale extracts. Likewise, growth in the white scale extract was about equal to that in the control, or better, while in the colored scale extracts it was decidedly retarded. In one experiment with *A. niger*, germination was inhibited by yellow scale extract and much reduced by the red scale ex-

tract. Where germination did occur, however, the growth was greater than in the control. In the second and third experiments the percentage of germination of *A. niger* was increased over the control in both white and colored scale extracts, and growth was practically the same in all three extracts.

It may be indicated, therefore, that whereas *Aspergillus* sp. 4660 is decidedly inhibited by the water extract from colored scales, *A. niger* is only moderately retarded, if at all. Hence no marked varietal resistance to the latter organisms would be expected in the colored types. This is in accord with the general observation.

ent symptoms and have been respectively designated gray mold neckrot, mycelial neckrot, and small sclerotial neckrot. All three organisms are in the main wound parasites and usually enter through the neck of the bulb. The first two species are active producers of decay, commonly invading the entire bulb. The third form is less aggressive and is more often confined to the outer scales.

The marked resistance of colored varieties of onion to Botrytis has long been recognized by onion growers and dealers. The greater susceptibility of white forms to *B. allii* is noted by Munn (*1*). Selby also mentions this fact (*2*), although it is not clear with

TABLE II.—*The effect of water extracts from dry outer scales of White, Yellow, and Red Globe varieties of onion on the germination and growth of certain species of Aspergillus and Helminthosporium as compared with Colletotrichum circinans*

Organism	Experiment No.	Germination in—				Length of germ tubes in—			
		Control	White scale extract	Yellow scale extract	Red scale extract	Control	White scale extract	Yellow scale extract	Red scale extract
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Microns*	*Microns*	*Microns*	*Microns*
Colletotrichum circinans	1	99	80	0	2	414	224	0	0
Aspergillus niger	1	38	97	0	8	11	55	0	40
	2	49	90	90	100				
	3	0	89	88	52	0	203	190	195
A. sp. 4660	1	88	98	1	0	47	38	1	0
	2	42	80	3	0				
	3	6	97	2	0	21	85	10	0
Helminthosporium sp.	1	84	100	0	2				

Helminthosporium sp. is a small-spored form which commonly causes dark-colored blotches on the outer scales of White Globe onions, but has never been noted on the colored forms. The toxic effect of colored scale extract is quite as distinct as with any other organism studied, while the white scale extract is apparently beneficial. It is probable, therefore, that the toxins in the red and yellow bulbs serve to restrict this organism largely to the white varieties.

RELATION OF PIGMENT TO DEVELOPMENT OF BOTRYTIS AND TO NECKROT RESISTANCE

Of the three species of Botrytis associated with the neckrot of onion, one, *Botrytis allii*, has been fully described by Munn (*1*). The remaining two species will be described in another paper by the senior writer, and will be referred to here as *Botrytis* sp. 110 and *Botrytis* sp. 108a. Although these forms commonly occur together, individually they cause somewhat differ-

which species of Botrytis he deals, since he undoubtedly was in error in referring to the causal organism as *Sclerotium cepivorum*. *Botrytis* sp. 110 and *Botrytis* sp. 108a are the commonest in the Wisconsin and Illinois onion-growing sections, and the writers' critical field observations are confined largely to these forms. In 1920 and 1923 white, red, and yellow onions were planted on old onion soil at Racine, Wis. The crops were placed in storage and the development of mycelial neckrot during that period noted.

The White Globe variety developed 56 per cent of mycelial neckrot in 1920 and 44 per cent in 1923. The Red Globe variety developed less than 1 per cent in both periods. Yellow Globe developed 6 per cent in 1920, but was not included in the 1923 test. Yellow Strasburg, not included in the test of 1920, developed less than 1 per cent in 1923.

The marked resistance of the colored varieties is thus evident. In both of these experiments comparative susceptibility to *Botrytis* sp. 108a was also

noted. A high percentage of the white bulbs was infected with this form in both years, while in no case was the fungus found on normally pigmented bulbs of the colored varieties. In 1920 the Yellow Globe seed used was not of a pure strain. A small number of the bulbs formed very little yellow pigment. *B.* sp. 108a developed on these, illustrating further the close correlation between pigment and resistance.

In spite of this marked expression of resistance, it is not uncommon to find cases where a fairly large percentage of colored bulbs are decayed with neckrot. That the extent of resistance to neckrot is limited is further substantiated by experiments in which spores and mycelium of *Botrytis allii* and *Botrytis* sp. 110 were injected through needle wounds into the succulent neck tissue of white, red, and yellow bulbs. Under such conditions the organisms were only slightly, if at all, exposed to the water-extractable toxins in the dry outer scales. In all of these experiments both organisms caused colored and white bulbs to decay with apparently equal rapidity. It is evident, therefore, that the resistance of colored bulbs to *B. allii* and *B.* sp. 110 is a resistance to initial infection, and where this is not prevented and the fungus becomes established in the succulent tissue all forms are equally susceptible.

Data secured from a study of the effect of outer-scale extracts upon germination and growth are of interest at this point. A strain of *Botrytis cinerea* isolated from cyclamen and nonpathogenic to onion was included in these tests. Table III shows that exposure to white scale extract had no detrimental effect on any of the forms tested; and in some instances the result was beneficial. In the case of yellow and red scale extracts the toxic effects are quite as marked as already noted for Fusarium, Aspergillus, Helminthosporium, and Colletotrichum. In two experiments with *B.* sp. 110 a high percentage of germination occurred in yellow scale extract, but it is to be noted that very meager growth resulted in these cases.

From the data just cited, resistance of colored bulbs is to be expected where the organisms are exposed to the toxins present in the outer colored scales. To what extent does this correlation exist in nature? The three forms of Botrytis parasitic on onion do not ordinarily attack the growing plants, but subsist as saprophytes until harvest time. As the tops of the onion mature, entrance is gained through the "neck" of the bulb. Comparable lots of White Globe bulbs, with the tops removed from one lot and left intact in the other, showed that in an appropriate environment the infection occurs as readily through an intact neck as through a wounded one. Therefore as the top dies down these organisms may be expected to invade the dying tissue at the neck and gradually enter the succulent tissue of the bulb. A considerable amount of moisture is necessary for growth at this point, as the senior writer has demonstrated (*5*) that *Botrytis* sp. 110, at least, can be almost completely checked by artificial drying of the neck tissues after incipient infection has taken place. In the necks of colored bulbs at this stage of maturity there is ordinarily a considerable quantity of intensely colored dead leaf or scale tissue. The extreme outer scales of colored bulbs usually develop little or no pigment at the

TABLE III.—*The effect of water extracts from dry outer scales of White, Yellow, and Red Globe varieties of onion on the germination and growth of several species of Botrytis as compared with Colletotrichum circinans*

Organism	Experiment No.	Germination in—				Length of germ tubes in—			
		Control	White scale extract	Yellow scale extract	Red scale extract	Control	White scale extract	Yellow scale extract	Red scale extract
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Microns*	*Microns*	*Microns*	*Microns*
Colletotrichum circinans	1	99	80	0	2	414	224	0	0
Botrytis allii	1	64	55	0	0	280	294	0	0
	2	97	94	4	4	491	554	5	28
	3	49	55	0	0				
B. sp. *110*	1	53	99	1	0	24	209	5	0
	2	53	100	94	0	24	82	10	0
	3	90	97	0	0				
	4	90	100	93	10				
B. sp. *108a*	1	100	100	4	14	341	839	27	39
	2	72	90	8	2	125	394	15	8
B. cinerea	1	93	96	0	3	143	166	0	7

neck and, as already noted (*4*), these are commonly affected with smudge during the latter part of the growing season. Nevertheless, some pigment develops in the underlying neck scales, and it becomes more intense with the desiccation of these tissues at maturity. The neckrot fungi entering at this latter period are thus exposed to the soluble toxins of dry colored scales, from which it is certain that the soluble toxins readily diffuse into any drops of moisture present.

It is believed that at this point the toxins associated with the scale pigments become the principal factor of resistance to Botrytis and serve as a barrier to infection. It is obvious that conditions are not always favorable to the expression of this resistant character. Frequently bulbs are harvested and the tops removed before complete maturity. This condition is unfavorable to the exclusion of the neckrot fungi, since it leaves fewer dry colored scales on the necks of colored bulbs and exposes a greater amount of succulent wound tissue, which is most conducive to the establishment of the fungus.

From this analysis of the question it may be assumed that the colored varieties of onion possess a quality of resistance to the three species of Botrytis causing the various types of neckrot. This resistance functions as a barrier to initial establishment of the fungi in the succulent tissues. Certain conditions of environment and handling of the crop may be expected to overthrow this means of defense at times. Resistance, therefore, in this case, as in most others, is not to be considered as absolute. There is, however, a definite substance (or group of substances) associated with pigmentation of the bulbs of colored varieties of onion which is toxic to the neckrot fungi, and this accounts in the main for the reputed resistance of these types to neckrot as compared with the well-known susceptibility of white varieties.

DISCUSSION OF RESULTS

Certain outstanding facts in the investigation to date of disease resistance in the onion may well be reviewed at this point. There exist in the onion tissue substances which, when extracted, exhibit a surprising amount of toxicity not only to fungi nonpathogenic to onion and to its mild parasites, but to its more aggressive parasites as well. Yet a fungus parasite is frequently found invading the tissue of the onion bulb, often in close proximity to these toxic substances or to substances from which the toxins are rapidly formed upon injury to the tissues. Therefore the mere presence of toxic substances in the host tissue does not necessarily imply resistance. The avenue of invasion and the device by which the organism parasitizes the host cell influence the ultimate effect which toxic substances within this cell may have upon the invader.

The studies on volatile onion oil (*6*) show that the toxicity in this case is general rather than specific to a given organism. Although the more rapidly invading bulb parasites are as a rule less sensitive to the volatile toxins than are the milder parasites, they do not ordinarily germinate or grow in the extracted onion juice, and are retarded materially by the volatile toxins from undiluted juice. Moreover, there is no correlation between the conspicuous resistance of colored varieties to smudge and neckrot and the amount of volatile toxins in the tissue. While it is postulated that the different degrees of retarding effect of the volatile toxins upon the different organisms may be responsible in part for the variation in parasitism of these organisms, this variation may be influenced by other factors as well, such as (1) mode of entrance, (2) method of attacking the host cell, (3) advance lethal effects of fungus enzymes, (4) food elements in the host.

The study of the range of toxicity of outer colored scale extract shows that this substance, as in the case of the volatile oil, is quite generally toxic to the fungi. With one notable exception (*Aspergillus niger*), the germination and growth of all of the organisms tested (those pathogenic and those nonpathogenic to onion) are decidedly inhibited in colored scale extracts, while they thrive in the extract of white scales. But here again the correlation between color and resistance is not general for all the bulb diseases of onion.

It would appear from the present studies that the toxic substance in colored scales becomes functional as a repellent principle in the dry outer bulb scales. There it diffuses readily into soil or meteoric water and inactivates the fungus, preventing its entrance into the living tissue of the underlying succulent scales. Consequently, those parasites which by nature of their respective channels of invasion come into direct contact with this toxic substance upon attacking the onion bulb would be expected to appear less frequently on colored than on white varieties.

This is strikingly true in the case of smudge (*Colletotrichum circinans*), where the parasite normally attacks at any point on the scale surface. In the case of black mold (*Aspergillus niger*) there is slight if any correlation between color and resistance. The organism attacks by almost precisely the same avenue as smudge, but the writers' tests have shown that the colored extracts have no consistent toxic effect upon this fungus. In the case of neckrot the entrance is usually through the neck of the bulb at about harvest time. Contacts with the toxins may be expected in a majority of cases, although as pointed out, this status may be changed under some conditions. Field observations show that there is a strong correlation between color and resistance to neckrot, but probably it is not so general as in the case of smudge.

Helminthosporium sp., which is essentially a saprophyte capable of attacking only the dead outer scales of onion bulb, has been found only on white varieties, but little is known as to its general occurrence. From its sensitiveness to the colored scale extract it is likely to be limited largely to the white varieties. There are probably other saprophytic soil organisms which occasionally develop on the outer scales of white varieties and which under certain conditions may act as mild parasites of the bulb in storage, but from which colored bulbs are protected by the repellent toxins in the outer scale.

The Fusarium bulb-rot organisms which invade most commonly through the stem plate, or through insect wounds, probably come under little or no influence of the toxins from the outer colored scales at the point of infection. Though they are very sensitive to these toxins, a strong expression of disease resistance would not logically be expected in this case. The limited evidence at hand points in this direction.

SUMMARY

(1) The marked resistance of colored types of onions to smudge (*Colletotrichum circinans*) is due to the presence in the outer scales of certain toxic substances closely associated with the pigment compounds or identical with them. The functioning of this resistant principle depends upon the ready diffusibility of these toxins from the dead outer scale into drops of meteoric or soil water, where they inactivate the fungus before it can attack the onion tissue. The purpose of the present investigation has been to determine the effect of these soluble toxins on certain other fungi, especially those which attack onion bulbs.

(2) The fungi considered, besides the smudge organism, were two onion-bulb-rotting species of Fusarium, namely, *F. cepae* Hanzawa and an unidentified form, referred to as *Fusarium* sp. 45; the tomato-wilt organism, *Fusarium lycopersici;* the wheat-scab organism, *Gibberella saubinetii* (*Fusarium graminearum*); the onion black-mold organism, *Aspergillus niger;* a garlic and onion bulb-decaying organism, *Aspergillus* sp. 4660; *Helminthosporium* sp. which occurs on the outer scales of white varieties; and three forms of *Botrytis allii*, Botrytis associated with onion neckrot, *Botrytis* sp. 110, and *Botrytis* sp. 108a.

(3) With one exception, *Aspergillus niger* (see below), the organisms germinated and grew quite as well in the extracts from dry outer white scales as in the control drops; but when extracts from dry outer colored scales were used, germination and growth were greatly retarded or entirely inhibited.

(4) The function of these toxins in rendering the bulb resistant to a given organism depends upon the organism's mode of attack.

(5) In the case of the onion Fusaria, which enter through insect wounds or through openings in the stem plate, there is little chance of contact with the soluble toxins in the outer scales. No evidence of varietal resistance correlated with pigmentation has been noted in this instance.

(6) *Aspergillus niger,* which normally attacks the outer scales, and which seems to be little affected by the outer scale toxins, develops quite as well on colored as on white varieties.

Helminthosporium sp., which is sensitive to the toxins, is evidently limited or restricted largely to the outer scales of white varieties.

(7) The neckrot fungi by their mode of entrance through the neck tissues ordinarily encounter in colored varieties a certain amount of the outer scale toxins. The generally observed escape of colored varieties from neckrot is probably due to this factor. More or less neckrot on colored varieties does occur in nature, however. Experimentally, it was shown that upon inoculation with *Botrytis allii* and *Botrytis* sp. 110 directly into the succulent tissue (thus eliminating the influence of the soluble toxins in the dry outer scales) infection occurred with approximately equal rapidity in colored and in white bulbs. It would appear therefore that the explanation for the occasional occurrence of neckrot epiphytotics in colored varieties will

be found in a sequence of particular conditions at harvest. One such condition is premature topping, which through wounds may expose succulent tissue to parasitic attack.

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ASEXUAL PROPAGATION AS AN AID TO THE BREEDING OF ROOTSTOCKS [1]

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It has been stated [2] that—

> nearly all plants may be propagated by cuttings from one or another of their parts. The ease with which plants may be multiplied in this way varies greatly in different species, and even in different varieties of the same species.
>
> Climate exerts a marked influence upon the tendency of plants to develop from cuttings. In certain localities in southern Europe and in parts of South America branches of the common apple tree, sharpened and driven into the ground as stakes, often take root and sometimes even bear fruit during the same season.

A comparatively warm soil and a cool atmosphere with abundant soil moisture are favorable to the rooting of cuttings. A coarse, sharp, clean sand has been recommended as the best material for use indoors, since it provides ample drainage and is comparatively free from the damping-off fungus. For cuttings which form roots readily, a mixture of one part of light garden loam to two parts of sand may be used. The cuttings should be kept in an environment sufficiently moist to prevent loss of water by evaporation and sufficiently warm to favor moderate root growth. The tops should be kept cool enough to prevent the early growth of leaves.

Cuttings of larger diameter root more readily than those of smaller diameter, which are more apt to shrivel and die. Wounds on the lower end of the cuttings start to heal by the production of a loose cellular mass of tissue known as a callus. The roots do not arise from the callus itself but from internal tissue. In many plants the roots bear no relation to the callus in position, as, for instance, in the figure shown in Plate 1, Group 3, C, where the roots are seen arising from the bark in the middle of the cutting. Bailey [3] states, however, that "as a matter of practice, best results are obtained from callused cuttings, particularly if the cuttings are made from mature wood, but this is probably due to the fact that considerable time is required for the formation of adventitious buds which give rise to the roots, not to any connection between the callusing and rooting processes themselves."

It has been the purpose of the preceding paragraphs to review briefly a few of the principles of the propagation of plants from cuttings in order that the results reported in this paper may be interpreted to better advantage. Let us now turn to a brief consideration of the desirability of asexual propagation of rootstocks from the standpoint of the breeding of improved types of pomological plants.

The breeding of improved pomological varieties has occupied the attention of horticulturists for a number of years. Most of this breeding work is of value for the number of varieties which have been produced rather than for an analysis of hereditary characters, as was pointed out by the writer in 1923.[4] This condition has resulted from the fact that long-life-cycled plants do not meet the requirements of favorable genetic material discussed by Babcock,[5] Malloch,[6] and others. The breeding of horticultural forms has been largely directed toward the improvement of the scion wood rather than of the rootstock. As soon as a desirable new type has been discovered, it has been propagated by asexual means, thus utilizing immediately the favorable morphological variations as well as any increased physiological vigor which the new type may possess. The breeding of scion wood has proceeded along these lines, however desirable a genetic analysis might be from the standpoint of future improvement.

The breeding of improved types of rootstocks has not received the attention it deserves, owing partly to the increased difficulty and expense of conducting such investigations and partly to the less obvious value of such endeavors. The more thoroughgoing

[1] Received for publication June 20, 1924—issued February, 1925.

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horticulturists recognize, however, the importance of the rootstock in its relation to soil conditions, to disease resistance, to uniformity of growth, and to the manner of union with the scion. In breeding for disease resistance it frequently happens that the resistance sought for resides in a distinct species. Such a species may or may not graft readily with the commercial type which it is desired to grow. It might be possible to unite the qualities of disease resistance and a possibility of grafting by hybridization. Such a hybrid would of course be heterozygous and would segregate in succeeding generations. In order to produce a desirable type for the propagation of rootstocks from seed it would be necessary to purify such a hybrid in succeeding generations. Such a procedure is costly both in time and resources. Add to this the possibility of sterility in the species cross, and the desirability of asexual propagation becomes more and more obvious.

Even when breeding is not for disease resistance, uniformity is desirable. In propagating rootstocks from seed, uniformity demands a homozygous parent tree. Relative homozygosity may be obtained in fruit trees with considerable expenditures, but at the present time our knowledge as to the purity of different forms is rather meager.

Hybrid vigor is utilized to considerable advantage by corn growers and it should be of as great advantage to pomologists. To utilize hybrid vigor of the first generation in fruit trees it is necessary to be able to propagate the hybrid by asexual means.

In order to test the relative merits of new types of scion wood derived from seedlings, they should be given uniform environmental conditions. Such uniformity as to rootstocks could be best secured by asexual propagation of the rootstock. Perhaps such a critical study of scion wood derived from seedlings which generally show great variability would not interest the practical breeder, but what about bud variations? Geneticists recognize two kinds of bud variations—first, modifications, and second, mutations. Modifications are ever present in fruit trees and are usually due to differences in combinations of environmental conditions, either internal or external, which existed during the development of that particular organ. Bud mutations, while comparatively rare, do occur in a large number of plants. Bud mutations are transmissible, while modifications are not. In order to determine whether we are dealing with a bud mutation or a modification, except in very striking cases, such as the production of red plums on the branch of a yellow plum tree, we must test out the variation to see whether or not it will come true to type. Such a test demands uniform rootstocks best secured by asexual propagation.

Cuttings of a large number of species and varieties were planted in February, 1923, to ascertain how many would root readily from cuttings. It was necessary to terminate the experiment in May, 1923. The cuttings were 1 foot long and from three-fifths to one-half inch in diameter. They were planted 10 inches deep, leaving about 2 inches exposed. The soil was a light loam mixed with a greenhouse soil consisting of peat, leaf mold, sand, manure, and loam. The soil was kept moist throughout the course of the experiment which was conducted on a well-drained piece of land in Berkeley, Calif. The cuttings were obtained from the University Farm, Davis, Calif., the United States Plant Introduction Gardens, Chico, Calif., the Citrus Experiment Station, Riverside, Calif., and the Connecticut Agricultural Experiment Station, Storrs, Conn. The accompanying table lists the number of cuttings of each sample which formed leaves, shoots, callus, roots, or made no growth. Some of the cuttings formed either a callus or roots in all of the forms listed in the table. A summary is given at the end of the table showing the species and varieties which failed to form either a callus or roots under the particular conditions of the experiment.

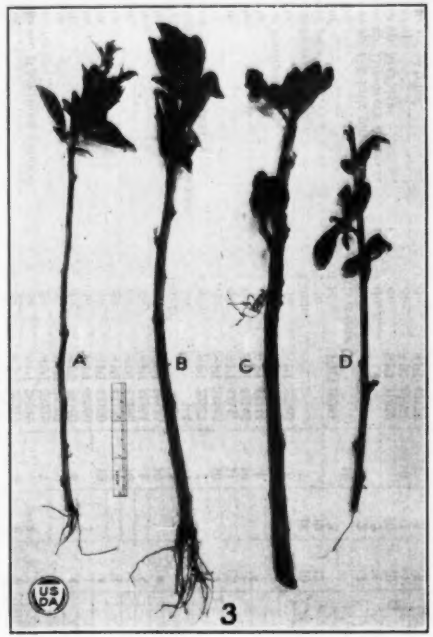
From the review given in the first part of the paper and from the description of the conditions of the experiment, the reader may conclude that experimental conditions were not

EXPLANATORY LEGEND FOR PLATE 1

Group 1:
A.—Rooted cuttings of *Prunus besseyi* from Connecticut.
B.—*Prunus munsoniana* from Connecticut.
C.—*Prunus pumila* from Connecticut.

Group 2:
A.—Rooted cuttings of *Cydonia oblonga*, SPI 33214.
B.—*Prunus bokhariensis*, SPI 40229.

Group 3:
A.—Rooted cuttings of Satsuma plum, Davis 13–12.
B.—Clyman plum, Davis 5–9.
C.—Hamari fig, SPI 6468.
D.—F_1 Strawberry × Peento, peach hybrid Davis, 1–12



A sexual Propagation in the Breeding of Rootstocks

(For explanatory legend see p. 516)

PLATE I

ideal in every respect for the rooting of cuttings. The soil, although well drained, would not, of course, be comparable to sand in this respect. It was not possible to supply bottom heat for the roots or to control atmospheric temperature. A number of the cuttings put forth leaves before a callus had formed, thus increasing transpiration. In view of these conditions the cuttings which did root are all the more interesting as examples of species or varieties which will root under slightly unfavorable environmental conditions. Under proper experimental conditions it might be possible to utilize asexual propagation to a far greater extent than is realized at present. It is with this thought in mind that the writer has submitted the data collected, hoping that others may continue the study beyond the point which has been possible in the present experiment.

After it has been demonstrated that a particular form will root readily from cuttings, it remains to be shown that such a rooted cutting will continue to develop and grow normally. The writer has not been able to continue the experiment to this stage. It is then necessary to select the particular form suited to the scion, to the soil, and to other environmental conditions. The Marianna type is known to root from cuttings, but it is open to the objection that it suckers freely in certain sections of the country. The Myrobalan plum is extensively used as a stock for different varieties of plums and prunes and can be grown from cuttings. There are several different types of Myrobalan plums and these will hybridize with certain other plums. Such hybrid seeds would probably cause a considerable variation in the resulting seedlings. The propagation of a desirable type of Myrobalan plum by asexual means would be a practical method of securing uniformity and a direct application of the ideas which the writer has tried to set forth in this paper. It is interesting to note that some nurserymen are actually doing this in California. It is hoped that the data presented in the accompanying table may be of some service to future investigators.

TABLE I.—*Showing data for the species and varieties of cuttings which formed either roots or a callus*[a]

Species and varieties	Source	Cutting No.	Leaves	Shoots	Callus	Roots	No growth
Chaenomeles lagenaria cathayensis	Chico	SPI 37954		3	3		
Do	do	SPI 46129	4		2		
Cydonia oblonga	do	SPI 32882		5	5		
Do	do	SPI 33214		5		5	
Ficus carica	do	PLH 6243	3			1	1
Archipel	do	SPI 18835	1			1	4
Constantine	do	SPI 18874	1		1		4
Hamari	do	SPI 6468	3			3	3
Maslin No. 20	do		2			1	3
Oeil de Perdrix	do	SPI 18842	1			1	4
Reculver	do	SPI 18868	2			2	3
Warren	do	SPI 18905	1			1	4
Xehba	do	SPI 6941	7			2	
Prunus amygdalus	do	SPI 7398	5		5		
Do	do	SPI 26543		3	3		
Do	do	SPI 33217	3	6	5		
Prunus sp. Buckthorn almond	do	SPI 28942		4	4		
Prunus persica	do	SPI 33219	7		1		
Do	do	SPI 36703	6		1		
Do	do	SPI 38469	4		4		
Do	do	SPI 40900		1	1		3
Do	do	SPI 43130	10		4		
Do	do	SPI 43133	10		4		
Do	do	SPI 43289	10		4		
Do	do	SPI 55564	8		8		3
Do	do	SPI 55835	10		4		
F_2 Strawberry × Peento	Davis	1–12	1			1	9
Family Favorite × Kalamazoo	do	1–4	1		1		9
Prunus persica nucipersica	Chico	SPI 29227	7		5		
Do	do	SPI 34685	10		4		
Do	do	SPI 43141	11		3	1	
Do	do	SPI 43142	10		1		
Do	do	SPI 43144	9		7		
Prunus fenzliana	do	SPI 35205	6		1		
Do	do	SPI 43303	8		1		
Prunus besseyi	Conn		7	18	10	8	
Prunus pumila	do			19	12	7	
Do	Chico	PIG 19261		3		3	
Do	Riverside		16			16	
Prunus sp. Gigantic plum	Chico			3	3		
Prunus sp. Discovery plum	do			2	1	1	
Prunus sp. Methley	do	SPI 31652		10	10		
Prunus sp.	do	SPI 32751	3	1	1		
Prunus sp. (Alpha)	do	SPI 43176		3	3		

TABLE I.—*Showing data for the species and varieties of cuttings which formed either roots or a callus*[a]—Continued

Species and varieties	Source	Cutting No.	Leaves	Shoots	Callus	Roots	No growth
Prunus sp. (Sharp Early)	Chico	SPI 43178		4	4		
Prunus sp. (Wright Purple)	do	SPI 43181		7	7		
Prunus sp. (Best Hybrid)	do	SPI 43182		14	12		
Prunus sp	do	SPI 47935	4		1		
Prunus armeniaca	do	SPI 26048	4		1		
Do	do	SPI 28961	3		1		
Do	do	SPI 28958	3		1		
Do	do	SPI 28959	2		2		
Do	do	SPI 28960	4		1		
Prunus bokhariensis	do	SPI 40231		4	4		
Do	do	SPI 40233	4		4		
Do	do	SPI 40229		4		3	
Do	do	SPI 40224		1	1		2
Prunus cerasifera (purple)	Riverside		20		10	8	
Prunus cerasifera	do		18		7		
Prunus cerasifera divaricata	Chico	SPI 37463		3	3		
Prunus cerasifera × *P. hortulana* (?) (The Marianna type)	Riverside			16		16	
Prunus dasycarpa	Chico			6	6		
Prunus domestica	do	SPI 30690		5	5		
Do	do	SPI 33224		4	4		
Do	do	SPI 30692		4	1		
Do	do	SPI 34268		7		7	
Prunus domestica (stock of Tribble Bros., Elk Grove, Calif.)	Riverside		15		15		
Agen (French Prune)	Chico			2	2		2
Do	Davis	8–13	10		5		
Clyman	do	5–9	7		2	5	3
Columbia	do	9–9	10		2		
Grand Duke	do	12–9	10		8		
Imperial Epineuse	do	4–15	10		2		
Italian Prune (Fellenberg)	Chico			5	1		
Peach Plum	Davis	13–8	8		6	1	1
Pond	do	11–9	10		3	2	
Sergeant	do	5–15	10		10		
Standard	Chico			5	5		
Sugar	do			4	4		
Do	Davis	2–15	10		10		
Sultan	do	12–13	2			1	4
Tragedy	do	8–9	8		1		2
Yellow Egg	do	7–10	10		2		
Prunus fremonti × (*Prunus cerasifera* ?)	Riverside			19		19	
Prunus lycioides	Chico	SPI 24808	4		4		
Do	do	SPI 28943	3		3		
Prunus mexicana	Conn			27	27		
Do	Riverside		32		17	1	
Prunus munsoniana	Conn			22	21	1	
Prunus spinosa	Riverside		12		2	2	
Prunus spinosa × *P. domestica*	Chico	SPI 32671		9	4		1
Do	do	SPI 32673		10	10		
Prunus salicina:							
Abundance	Davis	14–8	10		4		
Satsuma	do	13–12	10			10	
Santa Rosa	Chico			3	3		
Prunus salicina (?) × Combination	Davis	3–9	9		4	5	
Prunus salicina × *P. armeniaca* (Rutland Plumcot)	Chico			4		2	
Prunus salicina × *P. simonii*							
Wickson	Chico			1	1		
Climax	Davis	15–19	10		4		3
Prunus subcordata	Chico	SPI 32168	4		2		
Pyrus sp	do	SPI 26591		5	5		
Do	do	SPI 30308	3	1	1		
Do	do	SPI 37071		5	5		
Do	do	SPI 38799		4	1		
Do	do	SPI 44276		4	2		
Pyrus amygdaliformis	do	SPI 43754		5	5		
Pyrus betulaefolia	do	SPI 21982		5	5		
Pyrus calleryana	do	SPI 44006		6	6		
Pyrus communis	do	SPI 32736		4	4		
Do	do	SPI 32739		4	4		
Do	do	SPI 32745		4	4		
Do	do	SPI 32746		6	2		
Do	do	SPI 47093		3	3		
Favorita	do	SPI 33207		7	3		
Anjou	Davis	20–34	10		6		
Bartlett	do	20–20	10		8		
Bloodgood	do	20–26	10		6		
Clairgeau	do	20–29	10		8		
Clapp Favorite	do	20–15	10		5		
Colonel Wilder	do	20–43	9		7		
Colorado Seedless	do	20–14	10		3		
Comice	do	3–48	10		2		
Dana Hovey	Chico			3	3		
Do	Davis	20– 5	9		9		

TABLE I.—*Showing data for the species and varieties of cuttings which formed either roots or a callus*[a]—Continued

Species and varieties	Source	Cutting No.	Leaves	Shoots	Callus	Roots	No growth
Pyrus communis—Continued.							
Doyenne d'Alençon	Davis	20-31	9		5		
Easter Beurre	do	4-48	10		10		
Flemish Beauty	do	20-48	10		4		
Forelle	do	20-35	9		3		
Giffard	do	20-13	10		5		
Glou Morceau	do	20-28	10		10		
Hardy	do	20-19	10		7		
Do	do	20-40	10		4		
Howell	Chico			4	4		
Fox	Davis	20-52	8		5		
Kieffer	do	20- 4	10		8		
Lawson (Comet)	do	20-25	9		8		
P. Barry	do	20-41	10		6		
Seckel	do	20-37	10		3		
Summer Doyenne	do	20- 1	10		4		
Surprise	Chico	SPI 45901	2		2		
Winter Nelis	Davis	5-50	10		5		
Do	do	20- 9	10		6		
Pyrus nivalis	Chico	SPI 27670	3		1		
Pyrus phaeocarpa	do	SPI 39540	2	2	2		
Pyrus salicifolia	do	SPI 26680		3	3		
Pyrus serotina	do	SPI 30329		5		5	
Do	do	SPI 30352		5	5		
Do	do	SPI 30360		4	4		
Do	do	SPI 30361		4		2	
Do	do	SPI 38241		4	4		
Do	do	SPI 38242		3	3		
Do	do	SPI 38264	2	1	1		
Do	do	SPI 38271		5	1		
P. serotina × *P. communis*	do	SPI 43442		3		3	
Do	do	SPI 43443		5	3		
Do	do	SPI 43444		4	4		
Do	do	SPI 43562		4	4		
P. serotina (probably Golden Russet) × *P. communis*.	do	SPI 55805		4		4	
Pyrus ussuriensis	do	SPI 40019		6	6		
Do	do	SPI 44151		3	3		
Do	do	SPI 44150		5	5		
Pyrus sp. (Flowering crab)	do	SPI 22434		3	3		
Pyrus sp. (Muzalma)	do	SPI 30326	5		4		
Do	do	SPI 30326		5	2		
Do	do	SPI 30353		11	11		
Pyrus sp.	do	SPI 30635	5		2		
Do	do	SPI 30327	9		4		
Do	do	SPI 40207	6		6		
P. baccata × *P. malus*	do	SPI 28489		6	3		
Do	do	SPI 28489	4		1		
Pyrus malus	do	SPI 27152	4		1		
Do	do	SPI 27153	8		8		
Do	do	SPI 35636	3		1		
Do	do	SPI 39829	3		2		
Do	do	SPI 43154	2		1		
Do	do	SPI 43155	3		1		
Do	do	SPI 43157	17		9		
Do	do	SPI 43164	3		1		
Do	do	SPI 43168	3		2		
Do	do	SPI 43171	1		1		
Do	do	SPI 43173	3		2		
Pyrus malus:							
Chenango	Davis	8-44	10		10		
Cliff	Chico	SPI 43153	4		4		
Diadem	do	SPI 43157	4		1		
Early Harvest	Davis	7-45	10		10		
Gen. Carrington	Chico	SPI 43159	3		1		
Gravenstein	Davis	5-45	10		7		
John Sharp	Chico	SPI 43162	4		2		
Keswick	Davis	4-45	10		6		
Maiden Blush	do	8-44	10		8		
Red Astrachan	do	2-44	10		10		
Red June	do	9-45	10		1		
Red Spy	Chico	SPI 43169	4		2		
Summer Pearmain	Davis	10-45	10		7		
White Astrachan	do	1-48	10		10		
Willie Sharp	Chico	SPI 43174	3		3		
Yellow Transparent	Davis	2-50	10		3		
Pyrus pulcherrima (*P. floribunda*)	Chico	PIG 16625		7	7		
Pyrus sieboldi arborescens	do	SPI 43704		5	3		
Pyrus zumi	do	SPI 43705		6	6		3

[a] SPI refers to Seed and Plant Introduction numbers; PIG to Plant Introduction Garden numbers; and PLH to Plant Life History numbers.

The following failed to form either callus or roots: *Chaenomeles lagenaria cathayensis*, 2 varieties; *Crataegus pinnatifida*, 1 variety; *Ficus carica*, 79 varieties; *Prunus amygdalus*, 28 varieties; *P. persica*, 54 varieties; *P. persica* × *P. persica nucipersica*, 2 crosses; *P. persica nucipersica*, 5 varieties; *P.* subgenus *Amygdalus*, 8 species; *P. armeniaca*, 32 varieties; *P. avium*, 5 varieties; *P. cerasus*, 1 variety; *P. virginiana*, 3 varieties; *P. domestica*, 5 varieties; *P. salicina*, 4 varieties; *Prunus*, subgenus *Prunophora*, 23 species, 12 undetermined species; *Pyrus*, subgenus *Malus*, 5 species, 9 undetermined species; *P. malus*, 6 varieties; *P.* subgenus *Pyrus*, 2 species, 9 undetermined species; *P. serotina*, 13 varieties; *P. communis*, 8 varieties.

SUMMARY OF RESULTS

Species which formed roots and a callus: *Cydonia oblonga*, *Prunus besseyi*, *P. pumila*, *P. bokhariensis*, *P. cerasifera*, *P. fremonti* × *P. cerasifera* (?), *P. cerasifera* × *P. hortulana* (The Marianna type), *P. mexicana*, *P. munsoniana*, *P. spinosa*, *Prunus* sp. Discovery plum, *Pyrus serotina*, *P. serotina* × *P. communis*.

Species which formed a callus only: *Chaenomeles lagenaria cathayensis*, *Prunus fenzliana*, *P. dasycarpa*, *P. spinosa* × *P. domestica*, *P. lycioides*, *P. subcordata*, the following unnamed forms of Prunus, Gigantic plum, Methley, Alpha, Sharp Early, Wright Purple, Best Hybrid, SPI Nos. 32751, 47935; *Pyrus amygdaliformis*, *P. betulaefolia*, *P. calleryana*, *P. nivalis*, *P. phaeocarpa*, *P. salicifolia*, *P. ussuriensis;* the following unnamed forms of pears, SPI Nos. 26591, 30308, 37071, 38799, 44276; *Pyrus baccata* × *P. malus*, *P. pulcherrima*, *P. sieboldi arborescens*, *P. zumi*; and the following unnamed forms of apples, SPI Nos. 22434, 30326, 30353, 30635, 30327, 40207.

The following results were secured with commercial forms:

Varieties of *Ficus carica* which rooted: SPI 6243, Archipel, Hamari, Maslin No. 20 Oeil de Perdrix, Reculver, Warren, Xehba. Varieties of *Ficus carica* which formed a callus only: Constantine.

Varieties of *Prunus amygdalus* which formed a callus: SPI Nos. 7398, 26543, 33217, and *Prunus* sp. SPI No. 28942.

Varieties of *Prunus persica* which formed roots: F_1 Strawberry × Peento, *P. persica nucipersica* SPI 43141. Varieties of *P. persica* which formed a callus only, Family Favorite × Kalamazoo, SPI Nos. 33219, 36703, 38469, 40900, 43130, 43133, 43289, 55564, 55835; *P. persica nucipersica* SPI Nos. 29227, 34685, 43142, 43144.

Varieties of *Prunus armeniaca* which produced a callus only: SPI Nos. 26048, 28958, 28959, 28960, 28961.

Varieties of *Prunus domestica* which produced roots: SPI No. 34268, Clyman, Peach Plum, Pond, and Sultan. Varieties of *P. domestica* which produced a callus only: *P. domestica* stock of Tribble Bros., SPI Nos. 30690, 30692, 33224, Columbia, Italian Prune (Fellenberg), Agen (French Prune), Grand Duke, Imperial Épineuse, Sergeant, Standard, Sugar, Tragedy, Yellow Egg.

Varieties of *Prunus salicina* which produced roots: Satsuma, Combination and Rutland Plumcot. Varieties of *P. salicina* which produced a callus only: Abundance, Santa Rosa, Wickson and Climax.

Varieties of *Pyrus communis* which formed a callus only: SPI Nos. 32736, 32739, 32745, 32746, 47093, Favorita, Anjou, Bartlett, Bloodgood, Fox, Clairgeau, Clapp Favorite, Colonel Wilder, Colorado Seedless, Lawson (Comet), Comice, Dana Hovey, Doyenne d'Alençon, Summer Doyenne, Easter Beurre, Flemish Beauty, Forelle, Giffard, Glou Morceau, Hardy, Howell, Kieffer, P. Barry, Seckel, Surprise, Winter Nelis.

Varieties of *Pyrus malus* which formed a callus only: Chenango, Cliff, Diadem, Early Harvest, General Carrington, Gravenstein, John Sharp, Keswick, Maiden Blush, Red Astrachan, Red June, Red Spy, Summer Pearmain, White Astrachan, Willie Sharp, Yellow Transparent, and three plant introductions of the United States Department of Agriculture as follows: SPI Nos. 27152, 27153, 35636, 43154, 43155, 43157, 43164, 43168, 43171, 43173.

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